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August 17, 2000

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Re: Application of Mitsuyuki MATSUMOTO, Toru SUGIMOTO, Jun TAKASAKI, Masazumi KAMOHARA,  
Tetsu SAITO and Masato KOBAYASHI  
NOVEL G PROTEIN-COUPLED RECEPTOR PROTEINS  
Our Reference: Q60438  
PCT/JP99/01191, filed March 11, 1999

Dear Sir:

Applicants herewith submit the attached papers for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter II of the Patent Cooperation Treaty. Attached hereto is the application identified above which is a translation of PCT International Application No. PCT/JP99/01191, filed March 11, 1999, comprising the specification, claims, ten (10) sheets of drawings, executed Declaration and Power of Attorney, Sequence Listing, a 3.5" disk containing Sequence Listing in a computer readable form, Statement in Support of Submission in Accordance with 37 C.F.R. § 1.821, Notification Concerning Submission or Transmittal of Priority Document, International Preliminary Examination Report (foreign language), Preliminary Amendment, Amended Abstract, International Search Report, Information Disclosure Statement, PTO Form 1449 with reference, executed Assignment and PTO Form 1595.

The Government filing fee is calculated as follows:

Total Claims	12 - 20 =	0 x \$18 =	\$ 000.00
Independent Claims	2 - 3 =	0 x \$78 =	\$ 000.00
Base Filing Fee	(\$840.00)		\$ 840.00
Multiple Dep. Claim Fee	(\$260.00)		\$ 260.00
<b>TOTAL FILING FEE</b>			<b>\$1,100.00</b>
Recordation of Assignment Fee			\$ 40.00
<b>TOTAL U.S. GOVERNMENT FEE</b>			<b>\$1,140.00</b>

Checks for the statutory filing fee of \$ 1,100.00 and Assignment recordation fee of \$ 40.00 are attached. You are also directed and authorized to charge or credit any difference or overpayment to Deposit Account No. 19-4880. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. 1.492; 1.16 and 1.17 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Priority is claimed from:

Japanese Patent Application

P.Hei. 10-060245

P.Hei. 11-026774

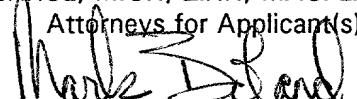
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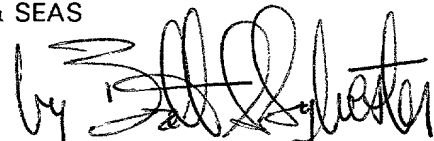
March 12, 1998

February 3, 1999

Respectfully submitted,  
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Q60438

Mitsuyuki MATSUMOTO, Toru SUGIMOTO, Jun TAKASAKI, Masazumi KAMOHARA, Tetsu SAITO and Masato KOBAYASHI

Serial No.: NOT YET ASSIGNED

PCT/JP99/01191, filed March 11, 1999

Filed: August 17, 2000

For: NOVEL G PROTEIN-COUPLED RECEPTOR PROTEINS

PRELIMINARY AMENDMENT

ATTN: BOX PCT

Assistant Commissioner for Patents  
Washington, DC 20231

Sir:

Prior to examination of the above-identified application, please amend the above-mentioned application as follows:

IN THE SPECIFICATION:

Page 30, line 7, please delete new paragraph break;

line 11, after "concentrations." please insert a new paragraph break.

REMARKS

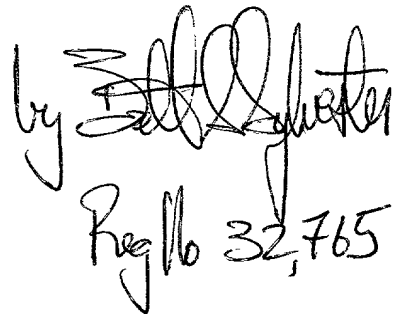
The above amendment(s) are made for editorial purposes.

Applicants submit no questions of new matter should arise and entry is requested.

Respectfully submitted,



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Description

Novel G Protein-coupled Receptor Proteins

Technical Field

This invention belongs to the genetic engineering field, and relates to novel G protein-coupled receptor proteins, genes coding for these G protein-coupled receptor proteins, methods for producing these G protein-coupled receptor proteins, screening methods using these G protein-coupled receptor proteins, antibodies for these G protein-coupled receptor proteins and screening methods using these antibodies.

Background Art

Cell membrane receptors which transmit signals to the intracellular region via the activation of heterotrimeric GTP binding protein are generally referred to as "G protein-coupled receptor". All members of the G protein-coupled receptor known to date are sometimes referred generally to as "seven transmembrane receptor", because they form a super family having a common structure which has the extracellular amino terminus and intracellular carboxyl terminus and passes through the cell membrane seven times. The G protein-coupled receptor transmits information on various physiologically active substances

from cell membranes to the intracellular region via activation of heterotrimeric GTP binding protein and subsequent changes in the intracellular second messengers induced. As the intracellular second messengers which are controlled by the heterotrimeric GTP binding protein, cAMP via adenylate cyclase,  $Ca^{++}$  via phospholipase C and the like are well known, and it has been revealed recently that many cellular proteins are their targets, such as the control of channels and activation of protein kinases via the heterotrimeric GTP binding protein (Gudermann, T. et al. (1997), *Annu. Rev. Neurosci.*, 20, 399 - 427). The physiologically active substances that transmit information via the G protein-coupled receptor include various known physiologically active substances such as neurotransmitters, hormones, chemokine, lipid-originated signal transducers, divalent ions and proteases. Information by these physiologically active substances is transmitted to the intracellular region through their specific G protein-coupled receptor, respectively.

Several hundred types of G protein-coupled receptor have so far been cloned from eucaryote. Regarding human, hundred or more types of G protein-coupled receptor for corresponding endogenous ligands have been cloned and are regarded as targets of drugs for diseases. There are various diseases in which G protein-coupled receptor is the

target, and there exist effective drugs which act upon G protein-coupled receptor, in the respective fields of central nervous system, circulatory organ system, inflammatory immune system, digestive organ system, motor organ system and urinary organ/reproductive organ system (Stadel, J. et al. (1997), *Trends Pharmacol. Sci.*, 18, 430 - 437). This indicates that agonists or antagonists of G protein-coupled receptor have a high possibility of becoming a therapeutic agent of diseases, so that studies are being actively carried out on the discovery and identification of new G protein-coupled receptors.

Cloning of G protein-coupled receptor genes tends to start based on their structural homology in the super family in many cases, and a receptor having no correspondence to endogenous ligand is referred to as "the orphan G protein-coupled receptor". In general, a ligand specific for the orphan G protein-coupled receptor has not been found, so that it was difficult to develop its agonist or antagonist. In recent years, however, it has been proposed to create a drug targeting for the orphan G protein-coupled receptor by combining the substantiated compound libraries and high performance high throughput screening (Stadel, J. et al. (1997), *Trends Pharmacol. Sci.*, 18, 430 - 437).

That is, it is possible to screen an agonist for an orphan G protein-coupled receptor from a compound library by effective high throughput system of the measurement of cAMP and  $Ca^{++}$  which are second messengers of many G protein-coupled receptors, or the measurement of GTPase activity and G protein binding of GTP $\gamma$ S which are indexes of the activation of heterotrimeric GTP binding protein, so that it is possible to find specific agonists and antagonists making use of such compounds and furthermore to develop therapeutic drugs for certain diseases. Under such conditions, discovery of a novel G protein-coupled receptor capable of becoming a new therapeutic target of diseases is regarded as the most important step in creating a medicament which acts upon G protein-coupled receptors.

Among G protein-coupled receptors, there is a case in which a plurality of receptors are present for one endogenous ligand. Such receptors are referred to as receptor family, and each receptor is called subtype. Since all of the G protein-coupled receptors have a common structure which passes through the cell membrane seven times, 20 to 25% of amino acids are preserved mainly in the transmembrane region even in mutually independent G protein-coupled receptors, but when they form a receptor family, ratio of the amino acids preserved among its subtypes significantly increases to 35% or more,

particularly to 60 to 80% among subtypes having high relevancy (Strader, C.D. et al. (1994), *Annu. Rev. Biochem.*, 63, 101 - 132).

When development of a therapeutic drug for diseases is planned by targeting for an endogenous ligand wherein a receptor family is present, specificity of its subtypes becomes important in many cases. This is because actions upon other subtype than actions upon a subtype that mediates the main action of a drug lead to side effects in many cases. Accordingly, it is desirable to create a subtype-specific agonist or antagonist, but it is necessary to find a means for detecting the subtype-specificity for that purpose. Currently, a method for constructing a system in which a gene of a subtype is cloned and its specificity is detected using a cultured cell line or the like which expresses the gene is generally used.

When a novel G protein-coupled receptor is used as the target of disease treatment, it is highly possible that the subtype-specificity is important, so that discovery of a receptor family is important also in the case of the novel G protein-coupled receptor. The homology of amino acid sequences among independent G protein-coupled receptors is 20 to 25% as a whole, but when they form a receptor family, the homology significantly increases in general in the family, so that it is possible to presume

whether they form a family or not, by comparing homology between two G protein-coupled receptors. It is possible to find novel G protein-coupled receptors which form a family, making use of such a means, and when a novel G protein-coupled receptor family is discovered, it will open a way for developing a drug for disease therapy because of the possibility of creating a subtype-specific agonist or antagonist.

The central nervous system transmits and controls various kinds of information using physiologically active substances represented by neurotransmitters. The G protein-coupled receptor is taking an important role in the signal transduction and control. Since many types of G protein-coupled receptor are present in the central nervous system, they are used as important therapeutic targets for diseases of the central nervous system. For example, it is considered that the G protein-coupled receptor of a neurotransmitter, dopamine, is a therapeutic target of schizophrenia (Seeman, P. et al. (1997), *Neuropsychopharmacology*, 16, 93 - 110), the G protein-coupled receptor of serotonin is that of depression (Cowen, P.J. (1991), *Br. J. Psychiatry*, 159 (Suppl. 12), 7 - 14), and the G protein-coupled receptor of neuro-peptide Y is that of eating disorder (Blomqvist, A.G. and Herzog, H. (1997), *Trends Neurosci.*, 20, 294 - 298).



It is considered that a novel G protein-coupled receptor expressing in the central nervous system, preferably a human receptor, will lead to a candidate for a new therapeutic target of central nervous system diseases or to the elucidation of central nervous system functions. In addition, for the purpose of developing a subtype-specific drug, it is desirable also to find a family in the case of the novel G protein-coupled receptor expressing in the central nervous system. Though the gene of a receptor GPR27 obtained from a mouse, having high homology with the amino acid sequence of SREB1 which is one of the G protein-coupled receptors of the invention, and an amino acid sequence based on its gene sequence have been reported (O'Dowd, B.F. et al. (1998), *Genomics*, 47, 310 - 313), no information is available to date concerning gene sequence and amino acid sequence of a human receptor.

#### Disclosure of the Invention

The present invention is to provide novel G protein-coupled receptor family proteins expressed in the central nervous system, as the target of therapeutic agents for central nervous system diseases.

With the aim of achieving the above object, the present inventors have conducted intensive studies and, as a result, succeeded in isolating genes (SREB1, SREB2,

SREB3, rSREB1, rSREB2 and rSREB3) which encode novel G protein-coupled receptor family proteins expressed in the central nervous system.

Also, we have established vectors containing these genes, host cells containing these vectors and methods for producing these G protein-coupled receptor proteins using such host cells, and rendered possible screening of these G protein-coupled receptor proteins and compounds, peptides and antibodies capable of modifying activities of the G protein-coupled receptor proteins.

Illustratively, the present invention relates to

(1) a G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26, or a G protein-coupled receptor protein as an equivalent to the protein,

preferably a human origin G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 2, 4 or 6 or a G protein-coupled receptor protein as an equivalent to the protein, or a rat origin G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 6, 22 or 26 or a G protein-coupled receptor protein as an equivalent to the protein,

(2) a G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26,

(3) a gene which has a nucleotide sequence coding for the G protein-coupled receptor protein described in the item (1),

(4) a vector which contains the gene described in the item (3),

(5) a host cell which contains the vector described in the item (4),

(6) a method for producing the G protein-coupled receptor protein described in the item (1) or (2), or a G protein-coupled receptor protein as an equivalent to the protein, which comprises using the host cell described in the item (5),

(7) a method for screening a medicament acting on the G protein-coupled receptor protein described in the item (1) or (2), which comprises allowing the G protein-coupled receptor protein to contact with a compound to be tested, or

(8) an antibody for the G protein-coupled receptor protein described in the item (1) or (2) or a partial peptide thereof.

The following explains the terms to be used herein.

The term "human origin" or "rat origin" means an amino acid sequence identical to the amino acid sequence of a G protein-coupled receptor protein expressing in human or rat.

The term "equivalent" of the G protein-coupled receptor protein of the present invention means a G protein-coupled receptor protein which is expressed in the central nervous system and shows the same activity of any one of the G protein-coupled receptor proteins represented by the amino acid sequences described in Sequence No. 2, 4, 6, 22 or 26.

In this connection, the G protein-coupled receptor and the G protein-coupled receptor protein have the same meaning.

The novel G protein-coupled receptor protein of the present invention is any one of the G protein-coupled receptor proteins represented by the amino acid sequences described in Sequence No. 2, 4, 6, 22 and 26, or equivalents thereof. Illustratively, all of G protein-coupled receptor proteins are included in the invention as long as they have the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26, or an amino acid sequence in which the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26, has substitution, deletion or insertion of one or a plurality, preferably from 1 to 10, more

preferably from 1 to 7, most preferably from 1 to 5, of amino acids, and have the same activity of the protein represented by the amino acid sequence described in Sequence No. 2, 4 or 6. Preferably, it is a human or rat origin G protein-coupled receptor protein having the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26.

Also, the gene which has a nucleotide sequence coding for the novel G protein-coupled receptor protein of the invention may be any gene, as long as it has a nucleotide sequence coding for the G protein-coupled receptor protein represented by the amino acid sequence described in Sequence No. 2, 4 or 6, or an equivalent thereof. Preferably, it is a gene which has a nucleotide sequence coding for the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26. More preferably, it is a gene which has a sequence of from 1 to 1,125 positions of the nucleotide sequence described in Sequence No. 1, from 1 to 1,110 positions of the nucleotide sequence described in Sequence No. 3, from 1 to 1,119 positions of the nucleotide sequence described in Sequence No. 5, from 1 to 1,131 positions of the nucleotide sequence described in Sequence No. 21, from 1 to 1,110 positions of the nucleotide sequence described in Sequence No. 23 or from 1 to 1,119 positions of the nucleotide sequence described in Sequence No. 25.

The gene which encodes the G protein-coupled receptor protein of the invention can be obtained by the following methods.

1) Production methods of novel G protein-coupled receptor protein gene

a) First production method

A mRNA sample is extracted from human cells or tissue having the ability to produce the G protein-coupled receptor protein of the invention. Next, using this mRNA as the template, two primers interposing the G protein-coupled receptor protein mRNA or a part of the mRNA region is prepared. The G protein-coupled receptor protein cDNA or a part thereof can be obtained by carrying out a reverse transcriptase-polymerase chain reaction (to be referred to as RT-PCR hereinafter) suited for SREB1, SREB2 or SREB3 by modifying the conditions for denature temperature, denaturing agent addition and the like. Thereafter, the receptor protein can be produced by integrating the thus obtained G protein-coupled receptor cDNA or a part thereof into an appropriate expression vector and expressing it in a host cell.

Firstly, mRNA molecules including those encoding the G protein-coupled receptor protein of the invention are extracted by a known method from cells or tissue, such as of the human brain or rat brain, having the ability to

produce the protein. Regarding the extraction method, a guanidine thiocyanate hot phenol method, a guanidine thiocyanate-guanidine hydrochloride method and the like can be exemplified, and a guanidine thiocyanate cesium chloride method can be cited as a preferred method. The cells or tissue having the ability to produce the protein can be identified by the Northern blotting method using a gene having a nucleotide sequence coding for the protein or a part thereof or by the Western blotting method using an antibody specific for the protein.

Purification of mRNA can be carried out in accordance with the conventional method, for example by adhering the mRNA to an oligo(dT) cellulose column and then eluting it therefrom. In addition, the mRNA can be further fractionated, for example, by a sucrose density gradient centrifugation. Alternatively, a commercially available already-extracted mRNA preparation may be used without carrying out the mRNA extraction.

Next, a single-stranded cDNA is synthesized from the thus purified mRNA by carrying out a reverse transcriptase reaction in the presence of a random primer or an oligo-dT primer. This synthesis can be carried out in the conventional way. The novel G protein-coupled receptor DNA of interest is amplified by subjecting the thus obtained single-stranded cDNA to PCR using two primers interposing a

region of the gene of interest. The thus obtained DNA is fractionated, for example, by an agarose gel electrophoresis. As occasion demands, a DNA fragment of interest can be obtained by digesting the DNA with restriction enzymes and then connecting the digests.

b) Second production method

In addition to the above method, the gene of the invention can also be produced making use of conventional genetic engineering techniques. Firstly, single-stranded cDNA is synthesized using the mRNA obtained by the above method as the template and a reverse transcriptase, and then double-stranded cDNA is synthesized from the single-stranded cDNA. Examples of the method include the S1 nuclease method (Efstratiadis, A. et al. (1976), *Cell*, 7, 279 - 288), the Land method (Land, H. et al. (1981), *Nucleic Acids Res.*, 9, 2251 - 2266), the O. Joon Yoo method (Yoo, O.J. et al. (1983), *Proc. Natl. Acad. Sci. USA*, 79, 1049 - 1053) and the Okayama-Berg method (Okayama, H. and Berg, P. (1982), *Mol. Cell. Biol.*, 2, 161 - 170).

Next, the recombinant plasmid obtained by the above method is introduced into an *Escherichia coli* strain, such as DH5 $\alpha$ , to effect its transformation, and a transformant can be selected making use of tetracycline resistance or ampicillin resistance as a marker. For example, when the host cell is *Escherichia coli*, transformation of the host



cell can be carried out by the Hanahan's method (Hanahan, D. (1983), *J. Mol. Biol.*, 166, 557 - 580), namely a method in which the recombinant DNA is added to competent cells prepared in the presence of  $\text{CaCl}_2$  and  $\text{MgCl}_2$  or  $\text{RbCl}$ . In this case, not only a plasmid but also a lambda or the like phage vector can also be used as the vector.

A strain having DNA coding for the novel G protein-coupled receptor protein of interest can be selected from the thus obtained transformants, for example by the following various methods.

(1) A screening method which uses a synthetic oligonucleotide probe

An oligonucleotide corresponding to the entire portion or a part of the G protein-coupled receptor protein of the invention is synthesized (in this case, it may be either a nucleotide sequence derived using the codon usage or a combination of plural possible nucleotide sequences, and in the latter case, their kinds can be reduced by including inosine), this is used as a probe (labeled with  $^{32}\text{P}$  or  $^{33}\text{P}$ ) and allowed to hybridize with DNA samples of transformants, which are denatured and fixed on a nitrocellulose filter, and then a positive strain is screened and selected.

(2) A screening method which uses a probe prepared by polymerase chain reaction

Sense primer and antisense primer oligonucleotides corresponding to a part of the G protein-coupled receptor protein of the invention are synthesized, and polymerase chain reaction (Saiki, R.K. et al. (1988), *Science*, 239, 487 - 491) is carried out using a combination of them to effect amplification of a DNA fragment of interest coding for the entire portion or a part of the G protein-coupled receptor protein. As the template DNA to be used herein, cDNA synthesized by the reverse transcription reaction from mRNA of cells capable of producing the G protein-coupled receptor protein or genomic DNA can be used. The thus prepared DNA fragment is labeled with  $^{32}\text{P}$  or  $^{33}\text{P}$  and used as the probe to select a clone of interest by carrying out colony hybridization or plaque hybridization.

(3) A screening method in which the novel G protein-coupled receptor protein is produced in other animal cells

A transformant is cultured to amplify the gene of interest, the gene is transfected into an animal cell (in this case, either a plasmid which can perform autonomous replication and contains a transcription promoter region or a plasmid which can be integrated into chromosome of the animal cell may be used) and a protein coded by the gene is produced on the cell surface. By detecting the protein

using an antibody specific for the G protein-coupled receptor protein of the invention, a strain of interest having cDNA coding for the G protein-coupled receptor protein is selected from the original transformants.

(4) A selection method which uses an antibody specific for the G protein-coupled receptor protein of the invention

In advance, cDNA is integrated into an expression vector and protein is produced on the surface of transformant strains, and then strains capable of producing the G protein-coupled receptor protein are detected using an antibody specific for the G protein-coupled receptor protein of the invention and a second antibody for the first antibody, thereby selecting a strain of interest.

(5) A method which uses a selective hybridization translation system

Samples of cDNA obtained from transformants are blotted on, for example, a nitrocellulose filter and hybridized with mRNA prepared from cells capable of producing the G protein-coupled receptor protein of the invention, and then the mRNA linked to the cDNA is dissociated and recovered. The thus recovered mRNA is then translated into protein using a protein translation system, for example by injecting into *Xenopus* oocyte or in a cell-free system such as a rabbit reticulocyte lysate, wheat germ or the like. A strain of interest is selected by

detecting it using an antibody for the G protein-coupled receptor protein of the invention.

Collection of DNA which encodes the G protein-coupled receptor protein of the invention from the thus obtained transformant of interest can be carried out in accordance with a known method (Maniatis, T. et al. (1982): "Molecular Cloning - A Laboratory Manual", Cold Spring Harbor Laboratory, NY). For example, it can be carried out by separating a fraction corresponding to a plasmid DNA from cells, and cutting out a cDNA region from the plasmid DNA.

c) Third production method

The gene which has a nucleotide sequence coding for the amino acid sequence represented by Sequence No. 2, 4, 6, 22 or 26 can also be produced by binding DNA fragments produced by a chemical synthesis method. Each DNA can be synthesized using a DNA synthesizer (e.g., Oligo 1000M DNA Synthesizer (Beckman), 394 DNA/RNA Synthesizer (Applied Biosystems) or the like).

d) Fourth production method

For the purpose of effecting expression of the function of G protein-coupled receptor protein of the invention by the substance thus obtained by genetic engineering techniques making use of the gene of the invention, it is not always necessary to have all of the amino acid sequences represented by Sequence No. 2, 4, 6,

22 and 26; for example, even if it is a partial sequence or other amino acid sequence is added thereto, such proteins are also included in the G protein-coupled receptor protein of the invention, as long as they show the same activity of the G protein-coupled receptor protein represented by the amino acid sequence shown in Sequence No. 2, 4, 6, 22 or 26. Also, as is known by the interferon gene and the like, it is considered that genes of eucaryote generally show polymorphism (e.g., see Nishi, T. et al. (1985), *J. Biochem.*, 97, 153 - 159), and there is a case in which one or a plurality of amino acid are substituted by this polymorphism or a case in which the nucleotide sequence is changed but the amino acids are completely unchanged. In consequence, even in the case of proteins in which one or a plurality of amino acid residues are substituted, deleted or inserted at one or a plurality of positions in the amino acid sequence represented by Sequence No. 2, 4 or 6, it is possible that they have the same activity of the G protein-coupled receptor represented by the amino acid sequence described in Sequence No. 2, 4 or 6. These proteins are called equivalents to the G protein-coupled receptor protein of the invention and included in the invention. In addition, a G protein-coupled receptor having the rat origin amino acid sequence shown by Sequence No. 22, 24 or

26 or a G protein-coupled receptor having the same activity of the former receptor is also included in the equivalents.

All of the genes having nucleotide sequences which encode these equivalents to the G protein-coupled receptor protein of the invention are included in the invention. Such various genes of the invention can also be produced by nucleic acid chemical synthesis methods in accordance with a usual method such as the phosphite triester method (Hunkapiller, M. et al. (1984), *Nature*, 10, 105 - 111), based on the information on the G protein-coupled receptor protein of the invention described in the foregoing. In this connection, codons for desired amino acid are well known, and they can be optionally selected and determined in the usual way, for example by taking codon usage of the host to be used into consideration (Crantham, R. et al. (1981), *Nucleic Acids Res.*, 9, r43 - r74). In addition, partial modification of codons of these nucleotide sequences can be carried out in the usual way in accordance, for example, with the site specific mutagenesis (Mark, D.F. et al. (1984), *Proc. Natl. Acad. Sci. USA*, 81, 5662 - 5666) which uses a primer comprised of a synthetic oligonucleotide coding for the desired modification.

Determination of the sequence of DNA obtained by the above methods a) to d) can be carried out by, for example, the Maxam-Gilbert chemical modification method (Maxam, A.M.

and Gilbert, E. (1980): "Methods in Enzymology", 65, 499 - 559) or the dideoxy nucleotide chain termination method (Messing, J. and Vieira, J. (1982), *Gene*, 19, 269 - 276) which uses M13.

Also, the vector of the invention, the host cell of the invention and the G protein-coupled receptor protein of the invention can be obtained by the following methods.

2) Production method of recombinant protein of the G protein-coupled receptor of the invention

An isolated fragment containing a gene coding for the G protein-coupled receptor protein of the invention can transform other eucaryotic host cell by again integrating into an appropriate vector DNA. In addition, it is possible to express the gene in respective host cells by introducing an appropriate promoter and a sequence related to the gene expression into these vectors.

Cells of vertebrates, insects, yeast and the like are included in the eucaryotic host cells and, though not particularly limited, examples of commonly used vertebrate cells include COS cell which is a simian cell (Gluzman, Y. (1981), *Cell*, 23, 175 - 182), a dihydrofolate reductase deficient strain of Chinese hamster ovary cell (CHO) (Urlaub, G. and Chasin, L.A. (1980), *Proc. Natl. Acad. Sci. USA*, 77, 4216 - 4220), human fetal kidney HEK293 cell and

293-EBNA cell (Invitrogen) prepared by introducing Epstein Barr virus EBNA-1 gene into the human cell.

As the expression vector for vertebrate cells, a vector which contains a promoter positioned on the upstream of the gene to be expressed, an RNA splicing site, a polyadenylation site, transcription termination sequence and the like can generally be used, and it may further contain a replication origin as occasion demands. Examples of the expression vector include pSV2dhfr having SV40 early promoter (Subramani, S. et al. (1981), *Mol. Cell. Biol.*, 1, 854 - 864), pEF-BOS having human elongation factor promoter (Mizushima, S. and Nagata, S. (1990), *Nucleic Acids Res.*, 18, 5322), pCEP4 having cytomegalovirus promoter (Invitrogen) and the like, though not limited thereto.

In a case in which COS cell is used as the host cell, an expression vector which has SV40 replication origin, can perform autonomous growth in COS cell and has a transcription promoter, a transcription termination signal and an RNA splicing site can be used, and its examples include pME18S (Maruyama, K. and Takebe, Y. (1990), *Med. Immunol.*, 20, 27 - 32), pEF-BOS (Mizushima, S. and Nagata, S. (1990), *Nucleic Acids Res.*, 18, 5322), pCDM8 (Seed, B. (1987), *Nature*, 329, 840 - 842) and the like. The expression vector can be incorporated into COS cell by, for example, the DEAE-dextran method (Luthman, H. and



Magnusson, G. (1983), *Nucleic Acids Res.*, 11, 1295 - 1308), the calcium phosphate-DNA co-precipitation method (Graham, F.L. and van der Ed., A.J. (1973), *Virology*, 52, 456 - 457), a method which uses FuGENE6 (Boeringer Mannheim) or the electroporation method (Neumann, E. et al. (1982), *EMBO J.*, 1, 841 - 845), and a desired transformant cell can thus be obtained.

Also, when CHO cell is used as the host cell, a transformant cell capable of stably producing the novel G protein-coupled receptor protein can be obtained by carrying out co-transfection of an expression vector together with a vector capable of expressing neo gene which functions as a G418 resistance marker, such as pRSVneo (Sambrook, J. et al. (1989): "Molecular Cloning - A Laboratory Manual", Cold Spring Harbor Laboratory, NY) or pSV2-neo (Southern, P.J. and Berg, P. (1982), *J. Mol. Appl. Genet.*, 1, 327 - 341), and selecting a G418 resistant colony. In addition, when 293-EBNA cell is used as the host cell, a desired transformant cell can be obtained using an expression vector which has Epstein Barr virus replication origin and can perform autonomous growth in the 293-EBNA cell, such as pCEP4 (Invitrogen).

The thus obtained desired transformant can be cultured in the conventional way, and the G protein-coupled receptor protein of the invention is produced inside the

cells or on the cell surface by this culturing. Regarding the medium to be used in this culturing, it can be optionally selected from various commonly used media depending on each host cell employed; for example, in the case of the COS cell, RPMI-1640 medium, Dulbecco's modified Eagle's minimum essential medium (DMEM) or the like can be used by adding serum components such as fetal bovine serum (FBS) and the like as occasion demands. Also, in the case of the 293-EBNA cell, Dulbecco's modified Eagle's minimum essential medium (DMEM) or the like medium supplemented with serum components such as fetal bovine serum (FBS) and the like can be used by further adding G418.

The G protein-coupled receptor protein of the invention thus produced inside the cell or on the cell surface of the transformant can be separated and purified therefrom by various known separation techniques making use of physical properties, chemical properties and the like of the receptor protein. Illustrative examples of such techniques, to be carried out after solubilization of the receptor protein-containing membrane fraction, include usual treatment with a protein precipitant, ultrafiltration, various liquid chromatography means such as molecular sieve chromatography (gel filtration), adsorption chromatography, ion exchange chromatography, affinity chromatography, high performance liquid

chromatography (HPLC) and the like, dialysis and combinations thereof. In this connection, the membrane fraction can be obtained in the usual way. For example, it can be obtained by culturing the cells which expressed the G protein-coupled receptor protein on the surface, suspending them in a buffer and then homogenizing and centrifuging them. Also, when the G protein-coupled receptor protein is solubilized using a solubilizing agent as mild as possible (CHAPS, Triton X-100, digitonin or the like), characteristics of the receptor can be maintained after the solubilization.

By effecting expression of the G protein-coupled receptor protein of the invention through its in-frame fusion with a marker sequence, confirmation of the expression the G protein-coupled receptor protein, confirmation of its intracellular localization, purification thereof and the like become possible. Examples of the marker sequence include FLAG epitope, Hexa-Histidine tag, Hemagglutinin tag, myc epitope and the like. Also, when a specific sequence recognizable by a protease such as enterokinase, factor Xa or thrombin is inserted between a marker sequence and the G protein-coupled receptor protein, the marker sequence can be cut and removed by such a protease. For example, there is a report in which muscarinic acetylcholine receptor and Hexa-

Histidine tag are connected with a thrombin-recognizing sequence (Hayashi, M.K. and Haga, T. (1996), *J. Biochem.*, 120, 1232 - 1238).

A method for the screening of compounds, peptides and antibodies capable of modifying activity of the G protein-coupled receptor protein is included in the invention. This screening method comprises adding an agent to be tested to a system in which an index of the modification of G protein-coupled receptor protein in response to a physiological characteristic of the G protein-coupled receptor protein is measured making use of the thus constructed G protein-coupled receptor protein, and measuring the index. The following screening methods can be cited as illustrative examples of this measuring system. Also, examples of useful drugs to be tested include compounds or peptides which are conventionally known to have G protein-coupled receptor ligand activity but their ability to selectively modify activity of the novel G protein-coupled receptor protein is not clear, known compounds and peptides registered in chemical files but their various G protein-coupled receptor ligand activities are unknown, compounds obtained by the method such as combinatorial chemistry techniques (Terrett, N.K. et al. (1995), *Tetrahedron*, 51, 8135 - 8137) and random peptides prepared by employing a phage display (Felici, F. et al.

(1991), *J. Mol. Biol.*, 222, 301 - 310) or the like. In addition, culture supernatants of microorganisms, natural components originated from plants and marine organisms, animal tissue extracts and the like are also objects of the screening. Also useful are compounds or peptides obtained by chemically or biologically modifying a compound or peptide selected by the screening method of the invention.

3) Screening methods of ligands of the G protein-coupled receptor protein of the invention, namely compounds, peptides and antibodies which modify activity of the G protein-coupled receptor protein of the invention

a) A screening method which uses a ligand binding assay method

Compounds, peptides and antibodies which bind to the G protein-coupled receptor protein of the invention (generally referred to as ligand) can be screened by a ligand binding assay method. A cell membrane sample obtained after expression of the receptor protein or a purified sample of the receptor protein is prepared, and a ligand purified for use in the ligand binding assay is radiation-labeled (50 to 2,000 Ci/mmol). Buffer solution, ions, pH and the like assay conditions are optimized, and the receptor protein-expressed cell membrane sample or the purified receptor protein sample is incubated in the thus

optimized buffer for a predetermined period of time together with the radiation-labeled ligand. After the reaction, this is filtered through, e.g., a glass filter and washed with an appropriate amount of the buffer, and then the radioactivity remained on the filter (total binding amount) is measured using, e.g., a liquid scintillation counter. Nonspecific binding amount is measured by adding the unlabeled ligand in large excess in the reaction solution, and the specific binding amount is obtained by subtracting the nonspecific binding amount from the total binding amount. A ligand showing specific binding to the receptor protein-expressed cell membranes or the purified receptor protein can be selected as a ligand of the G protein-coupled receptor protein of the invention. In addition, a compound, peptide or antibody having agonist activity, or a compound, peptide or antibody having antagonist activity, of the G protein-coupled receptor protein can be screened making use of the binding inhibition of the thus obtained radioactive ligand as an index.

b) A screening method which uses a GTPyS binding method

Compounds, peptides and antibodies capable of modifying the activity of the G protein-coupled receptor protein of the invention can be screened by a GTPyS binding

method (Lazareno, S. and Birdsall, N.J.M. (1993), *Br. J. Pharmacol.*, 109, 1120 - 1127). Cell membranes obtained after expression of the receptor protein is mixed with 400 pM of GTP $\gamma$ S labeled with <sup>35</sup>S in a solution of 20 mM HEPES (pH 7.4), 100 mM NaCl, 10 mM MgCl<sub>2</sub> and 50 mM GDP. After incubation in the presence or absence of an agent to be tested, this is filtered through, e.g., a glass filter and then radioactivity of the bound GTP $\gamma$ S is measured using, e.g., a liquid scintillation counter. A compound, peptide or antibody having agonist activity of the G protein-coupled receptor protein can be screened making use, as an index, of the increased specific GTP $\gamma$ S binding in the presence of the drug to be tested. Also, a compound, peptide or antibody having antagonist activity of the G protein-coupled receptor protein can be screened making use, as an index, of the suppression of increase in the GTP $\gamma$ S binding by the thus obtained compound, peptide or antibody having agonist activity.

c) A screening method which uses changes in the intracellular Ca<sup>++</sup> and cAMP concentrations

Many G protein-coupled receptor proteins induce increase in Ca<sup>++</sup> and/or increase or decrease in cAMP concentration in the cells caused by an agonist stimulus. Accordingly, compounds, peptides and antibodies capable of

modifying the activity of the G protein-coupled receptor protein of the invention can be screened making use of the changes in the intracellular  $\text{Ca}^{++}$  or cAMP concentration. The  $\text{Ca}^{++}$  concentration is measured using fura2 and the like, and the cAMP concentration is measured using a commercially available cAMP assay kit (by Amersham, etc.).

Alternatively, it is possible to measure the  $\text{Ca}^{++}$  and cAMP concentrations indirectly, by detecting the transcription activity of a gene whose transcription amount is controlled depending on the  $\text{Ca}^{++}$  and cAMP concentrations. A sample such as a compound, a peptide, a tissue extract or the like is allowed to react for a predetermined period of time with cells in which the receptor protein is expressed or host cells in which the receptor protein is not expressed (control cells), and the  $\text{Ca}^{++}$  and cAMP concentrations are measured directly or indirectly. A compound, peptide or antibody having agonist activity can be screened making use, as an index, of the increase in  $\text{Ca}^{++}$  and/or increase or decrease in cAMP concentration in the receptor protein-expressed cells by comparing with the control cells. Also, a compound, peptide or antibody having antagonist activity of the G protein-coupled receptor protein can be screened making use, as an index, of the increase in  $\text{Ca}^{++}$  and/or increase or decrease in cAMP concentration caused by the thus



obtained compound, peptide or antibody having agonist activity.

d) A screening method which uses Microphysiometer

Upon various signal responses of cells, trace amount of hydrogen ions outflow into the extracellular moiety is detected. Most of this outflow of hydrogen ions occur when metabolites formed by the fuel consumption of cells to obtain energy for their responses are increased or when signals of the cells are transmitted directly to the hydrogen ion pump. Since the G protein-coupled receptor protein of the invention requires energy for its signal transmission, outflow of hydrogen ions occurs when the receptor is activated. Since changes in pH caused by such a trace outflow of hydrogen ions in a medium around cells can be detected by CYTOSENSOR Microphysiometer (Molecular Devices), it can be used for the detection of the activation energy consuming receptors.

A compound, a peptide, a tissue extract or the like is allowed to react for a predetermined period of time with cells in which the receptor protein is expressed or host cells in which the receptor protein is not expressed (control cells), and changes in the pH due to outflow of hydrogen ions are measured. A compound, peptide or antibody having agonist activity can be screened making

use, as an index, of the changes in pH caused by the outflow of hydrogen ions from the receptor protein-expressed cells by comparing with the control cells. Also, a compound, peptide or antibody having antagonist activity of the G protein-coupled receptor protein can be screened making use, as an index, of the changes in pH due to the outflow of hydrogen ions caused by the thus obtained compound, peptide or antibody having agonist activity.

A medicament which contains as the active ingredient a compound, peptide or antibody capable of significantly modifying the activity of the G protein-coupled receptor protein or a G protein-coupled receptor protein selected by the screening method is included in the invention.

The antibody, such as a polyclonal antibody or monoclonal antibody, which reacts with the G protein-coupled receptor protein of the invention can be obtained by directly administering the novel G protein-coupled receptor protein or a fragment of the G protein-coupled receptor protein to various animals. It can also be obtained by a DNA vaccine method (Raz, E. et al. (1994), *Proc. Natl. Acad. Sci. USA*, 91, 9519 - 9523; Donnelly, J.J. et al. (1996), *J. Infect. Dis.*, 173, 314 - 320) using a plasmid in which a gene which encodes the G protein-coupled receptor protein of the invention is introduced.

The polyclonal antibody is produced from sera or eggs of an animal (e.g., rabbit, rat, goat, fowl or the like) immunized and sensitized by emulsifying the G protein-coupled receptor protein or a fragment thereof in an appropriate adjuvant such as complete Freund's adjuvant and administering it by intraperitoneal, subcutaneous or intravenous injection. The polyclonal antibody thus produced from sera or eggs can be separated and purified by the usual protein isolation purification methods. Examples of such methods include centrifugation, dialysis, salting out with ammonium sulfate, and chromatographic techniques using carriers such as DEAE-cellulose, hydroxyapatite, protein A agarose and the like.

An active antibody fragment containing a part of the antibody, such as  $F(ab')_2$ , Fab, Fab' or Fv, can be obtained by digesting the thus separated and purified antibody with a proteolytic enzyme such as pepsin, papain or the like in the usual way and subsequently separating and purifying it by the usual protein isolation purification methods.

It is possible for those skilled in the art to easily produce a monoclonal antibody by the cell fusion method of Kohler and Milstein (Kohler, G. and Milstein, C. (1975), *Nature*, 256, 495 - 497).

Mice are immunized by intraperitoneal, subcutaneous or intravenous inoculation of an emulsion prepared by

emulsifying the G protein-coupled receptor protein of the invention or a fragment thereof in an appropriate adjuvant such as complete Freund's adjuvant, several times repeatedly at intervals of several weeks. After final immunization, spleen cells are collected and fused with myeloma cells to prepare a hybridoma.

Myeloma cells having hypoxanthine-guanine phosphoribosyltransferase deficiency, thymidine kinase deficiency or the like marker, such as mouse myeloma cell strain P3X63Ag8.U1, are used as the myeloma cells for obtaining the hybridoma. Also, polyethylene glycol is used as the fusing agent. In addition, Eagle's minimum essential medium, Dulbecco's modified minimum essential medium, RPMI-1640 or the like generally used medium is optionally supplemented with 10 to 30% of fetal bovine serum and used as the medium for the preparation of the hybridoma. Fused strains are selected by the HAT selection method. Screening of hybridoma is carried out using a conventional method such as the culture supernatant by ELISA, immunohistological staining or the like or by the screening method described in the foregoing, and a hybridoma clone secreting the antibody of interest is selected. Also, monoclonal nature of the hybridoma is confirmed by repeating subcloning by means of limiting dilution analysis. When the thus obtained hybridoma is

cultured for 2 to 4 days in a medium or for 10 to 20 days in the abdominal cavity of a BALB/c mice pretreated with pristane, the antibody in an amount sufficient for purification is produced.

The thus produced monoclonal antibody can be separated and purified from the culture supernatant or ascites by the usual protein isolation purification methods. Examples of such methods include centrifugation, dialysis, salting out with ammonium sulfate, and chromatographic techniques using carriers such as DEAE-cellulose, hydroxyapatite, protein A agarose and the like. In addition, the monoclonal antibody or an antibody fragment containing a part thereof can also be produced by integrating entire portion or a part of a gene coding for the antibody into an expression vector and introducing into *Escherichia coli*, yeast or animal cells. An active antibody fragment containing a part of the antibody, such as F(ab')<sub>2</sub>, Fab, Fab' or Fv, can be obtained by digesting the thus separated and purified antibody with a proteolytic enzyme such as pepsin, papain or the like in the usual way and subsequently separating and purifying it by the usual protein isolation purification methods.

In addition, it is possible to obtain an antibody capable of reacting with the G protein-coupled receptor protein of the invention as single chain Fv or Fab by the

method of Clackson et al. or Zebedee et al. (Clackson, T. et al. (1991), *Nature*, 352, 624 - 628; Zebedee, S. et al. (1992), *Proc. Natl. Acad. Sci. USA*, 89, 3175 - 3179). It is also possible to obtain a human antibody by immunizing a transgenic mouse in which a mouse antibody gene is replaced by a human antibody gene (Lonberg, N. et al. (1994), *Nature*, 368, 856 - 859).

The medicament of the invention is characterized in that it has a novel pharmacological action to selectively control activity of the G protein-coupled receptor, and examples of the use of the medicament include central nervous system diseases which are induced by abnormalities of the G protein-coupled receptor activity (acceleration, reduction, denaturation and the like) or which express the abnormalities as complications.

The pharmaceutical preparation which contains a compound, peptide, antibody or antibody fragment capable of modifying activity of the G protein-coupled receptor protein of the invention, as the active ingredient, can be prepared using carriers, fillers and other additives generally used in the preparation of medicaments, in response to each type of the active ingredient.

Examples of its administration include oral administration in the form of tablets, pills, capsules, granules, fine granules, powders, oral solutions and the

like, and parenteral administration in the form of intravenous, intramuscular and the like injections, suppositories, percutaneous preparations, transmucosal absorption preparations and the like. Particularly, in the case of peptides which are digested in the stomach, intravenous injection or the like parenteral administration is desirable.

In the solid composition for use in the oral administration according to the present invention, one or more active substances are mixed with at least one inert diluent such as lactose, mannitol, glucose, microcrystalline cellulose, hydroxypropylcellulose, starch, polyvinyl pyrrolidone or aluminum magnesium metasilicate. In the usual way, the composition may contain additives other than the inert diluent, for example, a lubricant, a disintegrating agent, a stabilizing agent and a solubilizing or solubilization assisting agent. If necessary, tablets or pills may be coated with a sugar coating or a film of a gastric or enteric substance.

The liquid composition for oral administration includes emulsions, solutions, suspensions, syrups and elixirs and contains a generally used inert diluent such as purified water or ethanol. In addition to the inert diluent, this composition may also contain other additives

such as moistening agents, suspending agents, sweeteners, flavors and antiseptics.

The injections for parenteral administration includes aseptic aqueous or non-aqueous solutions, suspensions and emulsions. Examples of the diluent for use in the aqueous solutions and suspensions include distilled water for injection use and physiological saline. Examples of the diluent for use in the non-aqueous solutions and suspensions include propylene glycol, polyethylene glycol, plant oils (e.g., olive oil), alcohols (e.g., ethanol), polysorbate 80 and the like. Such a composition may further contain a moistening agent, an emulsifying agent, a dispersing agent, a stabilizing agent, a solubilizing or solubilization assisting agent, an antiseptic and the like. These compositions are sterilized for example by filtration through a bacteria retaining filter, blending of a germicide or irradiation. Alternatively, they may be used by firstly making into sterile solid compositions and dissolving them in sterile water or other sterile solvent for injection use prior to their use.

The dose is optionally decided by taking into consideration strength of each active ingredient selected by the screening method described in the foregoing and symptoms, age, sex and the like of each patient to be administered.



## Brief Description of the Drawings

Fig. 1 shows alignment of amino acid sequences of SREB1, SREB2 and SREB3.

Fig. 2 shows a result of Northern analysis of SREB1 in human organs.

Fig. 3 shows a result of Northern analysis of SREB1 in each region of human brain.

Fig. 4 shows a result of Northern analysis of SREB2 in human organs.

Fig. 5 shows a result of Northern analysis of SREB2 in each region of human brain.

Fig. 6 shows a result of Northern analysis of SREB3 in human organs.

Fig. 7 shows a result of Northern analysis of SREB3 in each region of human brain.

Fig. 8 shows a result confirming expression of SREB1, SREB2 or SREB3 protein.

Fig. 9 shows binding activity of anti-3L0 antibody for SREB1, SREB2 or SREB3.

Fig. 10 shows binding activity of anti-C24 antibody for SREB1.

Fig. 11 shows pCRE-Luc derived luciferase activity in cells in which SREB1, SREB2 or SREB3 was introduced.

Fig. 12 shows pSRE-Luc derived luciferase activity in cells in which SREB1, SREB2 or SREB3 was introduced.

#### Best Mode for Carrying Out the Invention

In order to disclose the invention further illustratively, Examples are described in the following, but the invention is not limited to these Examples. In this connection, unless otherwise noted, they can be carried out in accordance with known methods (Maniatis, T. et al. (1982): "Molecular Cloning - A Laboratory Manual", Cold Spring Harbor Laboratory, NY).

(Example 1) Isolation of genes coding for the novel G protein-coupled receptor family proteins

Full length cDNA coding for the G protein-coupled receptor family protein (SREB1, SREB2 or SREB3) of the invention was obtained by RT-PCR using human brain origin poly A<sup>+</sup> RNA (Clontech) as the template.

In the amplification of the novel G protein-coupled receptor human SREB1, 5'-AAAATCTAGA CGCGATGGCGAACGCGAGCGA-3' (Sequence No. 7) was used as the forward primer, and 5'-AAAATCTAGA GTCTATGTGGCGGGCCTCCC-3' (Sequence No. 8) as the reverse primer (XbaI site is added to each 5' terminus). RT-PCR was carried out using Pfu DNA polymerase (Stratagene) and by repeating a cycle of 98°C (20 seconds)/64°C (30 seconds)/74°C (3 minutes) 34 times in the

presence of 5% formamide. As the result, a DNA fragment of about 1.2 kbp was amplified. This fragment was digested with *Xba*I and then cloned using pCEP4 plasmid (Invitrogen). Since the pCEP4 plasmid contains CMV promoter which shows strong promoter activity in animal cells, it can be used in expressing recombinant proteins in animal cells. Nucleotide sequence of the thus obtained clone was analyzed by the dideoxy terminator method using ABI377 DNA Sequencer (Applied Biosystems). The thus revealed sequence is shown in Sequence No. 1 of the Sequence Listing.

This sequence contains an open reading frame of 1,125 bases (from the 1st position to the 1125th position of Sequence No. 1). An amino acid sequence (375 amino acids) deduced from the open reading frame is shown in Sequence No. 2 of the Sequence Listing. Since the deduced amino acid sequence contains seven hydrophobic regions considered to be the transmembrane domains which is a characteristic of the G protein-coupled receptor, it was found that this gene encodes the G protein-coupled receptor.

In the amplification of the novel G protein-coupled receptor human SREB2, 5'-AAAATCTAGA TCTATGGCGAACTATAGCCATGCA-3' (Sequence No. 9) was used as the forward primer, and 5'-AAAATCTAGA AAGGCTAAAGATTTACAGATGCTCC-3' (Sequence No. 10) as the reverse primer (*Xba*I site is added to each 5' terminus).

RT-PCR was carried out using Pfu DNA polymerase (Stratagene) and by repeating a cycle of 96°C (20 seconds)/54°C (30 seconds)/74°C (3 minutes) 34 times. As the result, a DNA fragment of about 1.2 kbp was amplified. This fragment was digested with *Xba*I and then cloned using pCEP4 plasmid (Invitrogen). Nucleotide sequence of the thus obtained clone was analyzed by the dideoxy terminator method using ABI377 DNA Sequencer (Applied Biosystems). The thus revealed sequence is shown in Sequence No. 3 of the Sequence Listing.

This sequence contains an open reading frame of 1,110 bases (from the 1st position to the 1110th position of Sequence No. 3). An amino acid sequence (370 amino acids) deduced from the open reading frame is shown in Sequence No. 4 of the Sequence Listing. Since the deduced amino acid sequence contains seven hydrophobic regions considered to be the transmembrane domains which is a characteristic of the G protein-coupled receptor, it was found that this gene encodes the G protein-coupled receptor.

In the amplification of the novel G protein-coupled receptor human SREB3, 5'-AAAATCTAGA GTATGGCCAACACTACCGGAGAG-3' (Sequence No. 11) was used as the forward primer, and 5'-AAAATCTAGA CCTGTCTGCCTACCAGCCTGC-3' (Sequence No. 12) as the reverse primer (*Xba*I site is added to each 5' terminus). RT-PCR

was carried out using Pfu DNA polymerase (Stratagene) and by repeating a cycle of 98°C (20 seconds)/62°C (30 seconds)/74°C (3 minutes) 34 times in the presence of 5% formamide. As the result, a DNA fragment of about 1.2 kbp was amplified. This fragment was digested with XbaI and then cloned using pCEP4 plasmid (Invitrogen). Nucleotide sequence of the thus obtained clone was analyzed by the dideoxy terminator method using ABI377 DNA Sequencer (Applied Biosystems). The thus revealed sequence is shown in Sequence No. 5 of the Sequence Listing.

This sequence contains an open reading frame of 1,119 bases (from the 1st position to the 1119th position of Sequence No. 5). An amino acid sequence (373 amino acids) deduced from the open reading frame is shown in Sequence No. 6 of the Sequence Listing. Since the deduced amino acid sequence contains seven hydrophobic regions considered to be the transmembrane domains which is a characteristic of the G protein-coupled receptor, it was found that this gene encodes the G protein-coupled receptor.

Homology of the novel G protein-coupled receptor SREB family (SREB1, SREB2 or SREB3) with a known G protein-coupled receptor family is 25% or less, respectively.

On the other hand, homology of SREB1 with SREB2 is 52%, homology of SREB1 with SREB3 is 52% and homology of SREB2 with SREB3 is 63%, which are significantly higher

than the homology with known G protein-coupled receptors (Fig. 1). This fact shows that the G protein-coupled receptors SREB1, SREB2 and SREB3 of the invention form a novel G protein-coupled receptor family independent of the known G protein-coupled receptors.

(Example 2) Expression distribution of human novel G protein-coupled receptor family genes in tissues

Expression distribution of the G protein-coupled receptor genes of the invention was analyzed by the northern blot hybridization method. A cDNA fragment (from the 722nd position to the 1054th position in Sequence No. 1) was used as the probe of human SREB1. Poly A<sup>+</sup> RNA (2 µg) originated from each of human organs was blotted on a membrane (Clontech), and its hybridization with the probe was carried out at 42°C (18 hours) in a solution containing 50% formamide, 5 x SSPE, 10 x Denhardt's solution, 2% SDS and 100 µg/ml denatured salmon sperm DNA. The membrane was finally washed twice (65°C for 30 minutes) with a solution containing 0.2 x SSC and 0.1% SDS. As shown in Fig. 2, when the northern analysis was carried out on each of human organs (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, large intestine and peripheral leukocyte), 3 kb of mRNA was detected in the brain, ovary, testis, heart and prostate, and 3 kb and 2.3 kb mRNA in the

peripheral leukocyte. Also, a signal of 3 kb was slightly detected in the pancreas, too. In addition, the northern analysis was also carried out on each of the regions of the human brain (amygdala, caudate nucleus, corpus callosum, hippocampus, substantia nigra, subthalamic nucleus, thalamus, cerebellum, cerebral cortex, medulla, spinal cord, occipital lobe, frontal lobe, temporal lobe and putamen). Since the 3 kb mRNA of the G protein-coupled receptor human SREB1 gene of the invention was detected in all of the examined human brain regions, it was found that it is expressed broadly in the human brain (Fig. 3).

A cDNA fragment (from the 558th position to the 888th position in Sequence No. 3) was used as the probe of human SREB2. When the northern analysis was carried out under the same conditions, 3.2 kb mRNA was detected in the brain, and 2.4 kb, 3.5 kb and 6.3 kb mRNA in the testis, as shown in Fig. 4. Also, the signal of 3.5 kb was detected in the placenta and spleen, and the signal of 3.2 kb in small intestine, all slightly. Among regions in the brain, the 3.2 kb mRNA of the G protein-coupled receptor human SREB2 gene of the invention was abundantly detected in the amygdala, caudate nucleus, hippocampus, substantia nigra, subthalamic nucleus, thalamus, cerebellum, cerebral cortexes and putamen, but not so much in the corpus callosum, medulla and spinal cord. In addition, a signal

of 7.8 kb was slightly detected in each of the grain regions (Fig. 5)

A cDNA fragment (from the 1st position to the 652nd position in Sequence No. 5) was used as the probe of human SREB3. When the northern analysis was carried out under the same conditions, 4 kb and 5.1 kb mRNA was detected in the brain, and 4 kb, 5.1 kb and 9.7 kb mRNA in the ovary, as shown in Fig. 6. The G protein-coupled receptor human SREB3 gene of the invention was detected in each region of the brain as signals of mainly 4 kb, 5.1 kb and slightly 9.7 kb, and the 4 kb mRNA was detected in the amygdala, hippocampus, subthalamic nucleus, cerebellum and cerebral cortex, and the 5.1 kb mRNA in the substantia nigra, subthalamic nucleus and spinal cord, relatively abundantly (Fig. 7).

The above results showed that the G protein-coupled receptor family genes SREB1, SREB2 and SREB3 of the invention are expressed mainly in the central nervous system and urinary organ/reproductive organ system.

(Example 3) Confirmation of the expression of the novel human G protein-coupled receptor family proteins

pCEP4 (Invitrogen) was used as the expression vector for expressing human SREB1, SREB2 or SREB3. In this case, in order to fuse a FLAG epitope as a marker sequence with the N terminus of human SREB1, SREB2 or SREB3,



ATGGACTACAAGGACGACGATGACAAGGGGATCCTG (Sequence No. 13) was inserted into the 5' terminus of the protein coding sequence of SREB1, SREB2 or SREB3. The thus constructed plasmids were named pCEP4-FL-SREB1, pCEP4-FL-SREB2 and pCEP4-FL-SREB3, respectively. By the use of these plasmids, a polypeptide in which a sequence Met Asp Tyr Lys Asp Asp Asp Asp Lys Gly Ile Leu (Sequence No. 14) is fused with the N terminus of the polypeptide of SREB1, SREB2 or SREB3 is expressed.

A  $1 \times 10^6$  cells portion of 293-EBNA (Invitrogen) was inoculated into a 10 cm Petri dish and cultured for 1 day, and then gene transfer of 8  $\mu$ g of pCEP4-FL-SREB1, pCEP4-FL-SREB2, pCEP4-FL-SREB3 or pCEP4-FL (vector alone) was carried out using FuGENE6 (Boeringer Mannheim). After the gene transfer, the cells were cultured for 1 day, harvested, washed, suspended in 20 mM of Tris-HCl (pH 7.4)/150 mM NaCl/Complete™ (Boeringer Mannheim) and then homogenized using Polytron. The homogenate was mixed with Triton X-100, Digitonin and sodium cholate to final concentrations of 0.2%, 0.1% and 0.2% and then solubilized by incubating at 4°C for 2 hours. Immunoprecipitation of the FLAG epitope fusion protein from the thus solubilized sample was effected using M2-agarose (Sigma). The immune precipitate was eluted with 200  $\mu$ M FLAG peptide/20 mM Tris-HCl (pH 7.4)/150 mM NaCl. The eluted sample was

concentrated, subjected to electrophoresis using SDS/10%-20% acrylamide gel (Daiichi Pure Chemicals) and then transferred on a PVDF membrane using a blotting apparatus. The PVDF membrane after the transfer was subjected to blocking and then allowed to react with a mouse anti-FLAG monoclonal antibody (M2; Sigma) and a horseradish peroxidase-labeled rabbit anti-mouse IgG polyclonal antibody (Zymed) in that order. After the reaction, expression of SREB1, SREB2 or SREB3 protein was confirmed using ECL Western Blotting Detection System (Amersham-Pharmacia) (Fig. 8).

The protein capable of reacting with the anti-FLAG antibody was not present in the cells in which pCEP4-FL was introduced but detected in the cells in which pCEP4-FL-SREB1, pCEP4-FL-SREB2 or pCEP4-FL-SREB3 was introduced as a band of 35 to 45 kDa. Estimated molecular weights of human SREB1, human SREB2 and human SREB3 were 39.8 kDa, 42.0 kDa and 41.5 kDa, respectively, and their bands were found at positions of almost expected molecular weights. In addition, a band of 65 to 75 kDa considered to be a dimer was detected in the case of human SREB1.

(Example 4) Isolation of gene coding for rat SREB1

(rSREB1), rat SREB2 (rSREB2) or rat SREB3 (rSREB3) protein

Complete length cDNA coding for rSREB2, rSREB2 or rSREB3 was obtained by RT-PCR using rat brain origin poly A<sup>+</sup> RNA (Clontech) as the template.

In the amplification of rSREB1, 5'-AAAATCTAGACGGCGATGGCGAACGCTAGTGA-3' (Sequence No. 15) was used as the forward primer, and 5'-AAAATCTAGACACTTTGAGAGTCTTGTGAAGGC-3' (Sequence No. 16) as the reverse primer (XbaI site is added to each 5' terminus). Amplification, cloning and nucleotide sequence determination of cDNA were carried out by the same methods of Example 1. The thus revealed sequence is shown in Sequence No. 21 of the Sequence Listing.

This sequence contains an open reading frame of 1,131 bases (from the 1st position to the 1131st position of Sequence No. 21). An amino acid sequence (377 amino acids) deduced from the open reading frame is shown in Sequence No. 22 of the Sequence Listing. Since the deduced amino acid sequence coincided in 97% frequency with the human SREB1, it was found that this gene encodes rSREB1.

In the amplification of rSREB2, 5'-AAAATCTAGATCTATGGCGAACTATAGCCATGC-3' (Sequence No. 17) was used as the forward primer, and 5'-AAAATCTAGAAAGGCTAAAGATTTACAGATGCTCC-3' (Sequence No. 18) as the

reverse primer (XbaI site is added to each 5' terminus). Amplification, cloning and nucleotide sequence determination of cDNA were carried out by the same methods of Example 1. The thus revealed sequence is shown in Sequence No. 23 of the Sequence Listing.

This sequence contains an open reading frame of 1,110 bases (from the 1st position to the 1110th position of Sequence No. 23). An amino acid sequence (370 amino acids) deduced from the open reading frame is shown in Sequence No. 24 of the Sequence Listing. Since the deduced amino acid sequence coincided in 100% frequency with the human SREB2, it was found that this gene encodes rSREB2.

In the amplification of rSREB3, 5'-AAAATCTAGACAAATACTGAACTGGCCGATCCCC-3' (Sequence No. 19) was used as the forward primer, and 5'-AAAATCTAGATGTTGGCCCCAGTATGGTGATCAT-3' (Sequence No. 20) as the reverse primer (XbaI site is added to each 5' terminus). Amplification, cloning and nucleotide sequence determination of cDNA were carried out by the same methods of Example 1. The thus revealed sequence is shown in Sequence No. 25 of the Sequence Listing.

This sequence contains an open reading frame of 1,119 bases (from the 1st position to the 1119th position of Sequence No. 25). An amino acid sequence (373 amino acids) deduced from the open reading frame is shown in Sequence

No. 26 of the Sequence Listing. Since the deduced amino acid sequence coincided in 99% frequency with the human SREB3, it was found that this gene encodes rSREB3.

(Example 5) Preparation of antibody for human SREB1

A partial amino acid sequence of human SREB1 was fused with glutathione-S-transferase (GST) and used as the immunization antigen for the preparation of antibody for human SREB1. Illustratively, a cDNA fragment corresponding to a region of from the 208th position to the 282nd position (3LO) and a region of from the 351st position to the 375th position (C24) of the human SREB1 amino acid sequence (Sequence No. 2) was amplified by PCR in an way to bind cleavage sites of restriction enzymes *Bam*HI and *Xho*I, and inserted between *Bam*HI and *Xho*I sites of GST Gene Fusion Vector (pGEX-5X-1: Amersham-Pharmacia). Competent cells of an *Escherichia coli* strain BL21(DE3)pLysS (Novagen) were transformed with the thus constructed plasmid. By culturing the transformant and inducing expression of the gene with 1 mM IPTG, a GST-3LO fusion protein and a GST-C24 fusion protein were expressed in the *E. coli* cells. The GST-3LO and GST-C24 were purified from disrupted *E. coli* cells using Glutathione Sepharose 4B (Amersham-Pharmacia) in accordance with the instruction attached thereto.

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The thus purified GST-3LO fusion protein was mixed with the same amount of Freund's complete adjuvant (CalBioChem) and emulsified, and the emulsion was administered to a female white Leghorn (140 days of age) at around the bursa of Fabricius. The initial dose was 1 mg, and it was administered thereafter in 0.5 mg portions 4 times at intervals of 2 weeks. After the final immunization, eggs were collected, the yolk of eggs was diluted with physiological saline and defatted using dextran sulfate, and then IgY was purified using DEAE Sepharose (Amersham-Pharmacia) to obtain anti-3LO antibody. Also, the purified GST-C24 fusion protein was mixed with the same amount of TiterMax Gold (CytRX) and emulsified, and the emulsion was administered under the dorsal skin of a Japanese white rabbit (6 weeks of age). Its initial dose was 1 mg, and it was administered thereafter in 0.5 mg portions 2 times at intervals of 2 weeks. After the final immunization, blood was collected, and IgG was purified from the serum using Protein G Sepharose (Amersham-Pharmacia) in accordance with the instruction attached thereto, thereby obtaining anti-C24 antibody.

Since the anti-3LO antibody uses a region of from the 208th position to the 282nd position of the human SREB1 amino acid sequence (Sequence No. 2) as the antigen and this partial amino acid sequence contains a large number of

sequences common to SREB1, SREB2 and SREB3 (cf. Fig. 1), there is a possibility that the anti-3LO antibody commonly recognizes SREB1, SREB2 and SREB3. Also, since the anti-C24 antibody uses a region of from the 351st position to the 375th position of the human SREB1 amino acid sequence (Sequence No. 2) as the antigen and this partial amino acid sequence is a sequence in which SREB2 and 3 are not present but SREB1 alone is present (cf. Fig. 1), there is a possibility that the anti-C24 antibody recognizes only SREB1. In consequence, in order to confirm the specificity of anti-3LO antibody and anti-C24 antibody, Western blotting was carried out using the immune precipitate of anti-FLAG antibody of 293-EBNA in which SREB1, SREB2 or SREB3 was expressed, prepared in Example 3, and the anti-3LO antibody and anti-C24 antibody.

Illustratively, each sample was subjected to electrophoresis using SDS/10%-20% acrylamide gel (Daiichi Pure Chemicals) and then transferred on a PVDF membrane using a blotting apparatus. The PVDF membrane after the transfer was subjected to blocking and then allowed to react with 10 µg/ml of the anti-3LO antibody and a horseradish peroxidase-labeled rabbit anti-chicken IgG polyclonal antibody (Zymed) in that order or with 10 µg/ml of the anti-C24 antibody and a horseradish peroxidase-labeled goat anti-rabbit IgG polyclonal antibody (MBL) in

that order. After the reaction, color development was carried out using ECL Western Blotting Detection System (Amersham-Pharmacia). A band reacting with the anti-3LO antibody was detected at the same position of the anti-FLAG antibody of Example 3 in cells in which pCEP4-FL-SREB1, pCEP4-FL-SREB2 or pCEP4-FL-SREB3 (Fig. 9) was introduced. Also, a band reacting with the anti-C24 antibody was detected at the same position of the anti-FLAG antibody of Example 3 only in the cells in which pCEP4-FL-SREB1 was introduced (Fig. 10).

Based on the above results, it was confirmed that the anti-3LO antibody is an antibody which recognizes SREB1, SREB2 or SREB3, and the anti-C24 antibody is an antibody which recognizes only SREB1. The use of these antibodies has rendered possible the detection of natural SREB1, SREB2 or SREB3 by the method such as the Western blotting, immunohistological staining or the like.

(Example 6) Evaluation of transcription activity via cAMP-response element (CRE) or serum response element (SRE) in human SREB1-, SREB2- or SREB3-introduced cells

Increase in the transcription activity mediated by CRE or SRE is induced by the activation of the intracellular information transmission system of various G protein-coupled receptors (Lolait, S.J. *et al.* (1992),



*Nature*, 357, 336 - 339; Hoeltzel, W.L. et al. (1997), *Am. J. Physiol.*, 273, C2037 - C2045; An, S. et al. (1998), *J. Biol. Chem.*, 273, 7906 - 7910). Also, it is known that the intracellular information transmission system of G protein-coupled receptors is partially activated via a certain transitional active conformation even in the absence of agonist (Kenakin, T. (1995), *Trens. Pharmacol. Sci.*, 16, 188 - 192). Accordingly, if changes in the CRE- or SRE-mediated transcription activity in SREB1-, SREB2- or SREB3-introduced cells are found even in the absence of agonist, it can be confirmed that the G protein-coupled receptor is functional and that activation of the G protein-coupled receptor intracellular information transmission system leads to the CRE- and SRE-mediated transcription activity.

Using pEF-BOS (Mizushima, S. and Nagata, S. (1990), *Nucleic Acids Res.*, 18, 5322) as the expression vector for expressing human SREB1, SREB2 or SREB3, pEF-BOS-SREB1, pEF-BOS-SREB2 and pEF-BOS-SREB3 were prepared. A  $8 \times 10^4$  cells portion of 293-EBNA (Invitrogen) was inoculated into a 24-well plate and cultured for 1 day, and then gene transfer of 250 ng of pEF-BOS-SREB1, pEF-BOS-SREB2, pEF-BOS-SREB3 or pEF-BOS (vector alone) was carried out together with 25 ng of a CRE-reporter plasmid pCRE-Luc (Stratagene) or an SRE-reporter plasmid pSRE-Luc (Stratagene), using FuGENE6 (Boeringer Mannheim) (3 wells for each). After the gene

transfer, the cells were lysed at every 12 hours using PicaGene Cell Culture Lysis Reagent Luc (Nippon Gene), and the activity of luciferase produced from each reporter plasmid was measured using PicaGene Luminescence Kit (Nippon Gene).

The luciferase activity in the SREB1-, SREB2- or SREB3-introduced cells after 24 hours of the gene transfer was treated as a relative activity to the luciferase activity of the vector alone introduced cells (control) (the control was defined as 1), with the results shown in Fig. 11 (pCRE-Luc derived luciferase activity) and Fig. 12 (pSRE-Luc derived luciferase activity). The CRE-mediated transcription activity increased most sharply in the SREB1-introduced cells and also increased significantly in the SREB2- and SREB3-introduced cells in comparison with the control. On the other hand, the SRE-mediated transcription activity increased most sharply in the SREB2-introduced cells and also increased significantly in the SREB1- and SREB3-introduced cells in comparison with the control.

It was revealed by these results that the SREB1, SREB2 and SREB3 are functional receptors, and activation of the intracellular information transmission system of these G protein-coupled receptors leads to the increase in the CRE- or SRE-mediated transcription activity.

## Industrial Applicability

Novel G protein-coupled receptor family proteins SREB1, SREB2 and SREB3 expressing in the central nervous system, genes coding for these proteins, vectors containing these genes, host cells containing these vectors and methods for producing these G protein-coupled receptor proteins were provided by the present invention.

Also, it rendered possible to screen new medicaments, particularly new therapeutic agents for central nervous system diseases, through the screening of compounds, peptides and antibodies capable of modifying activities of the G protein-coupled receptor proteins of the invention by allowing the G protein-coupled receptors to contact with drugs to be tested.

Regarding the medicament of the invention which contains, as the active ingredient, a compound, peptide or antibody capable of specifically modifying activity of the G protein-coupled receptor proteins expressing in the central nervous system, its usefulness as therapeutic agents and the like for functional/organic diseases of the central nervous system. Also, since the G protein-coupled receptor family proteins of the invention are expressed not only in the central nervous system but also in the urinary organ/reproductive organ system, usefulness as therapeutic drugs and the like for diseases related to the urinary

organ/reproductive organ system can be expected from the medicament which contains, as the active ingredient, a compound, peptide or antibody capable of specifically modifying their activities. In addition, since a member of the G protein-coupled receptors of the invention, such as SREB1 protein, is expressed not only in the central nervous system and urinary organ/reproductive organ system but also in the heart and peripheral leukocytes, a medicament which contains, as the active ingredient, a compound, peptide or antibody capable of specifically modifying the activity of SREB1 protein can be expected for its usefulness as therapeutic drugs and the like for circulatory system diseases and immune inflammation system diseases, in addition to central diseases and diseases related to the urinary organ/reproductive organ system.

The novel G protein-coupled receptor family SREB1, SREB2 or SREB3 of the invention has markedly high conservation ratio of amino acids in human and rat. This conservation ratio is most highest among the existing G protein-coupled receptor families, which seems to show that the novel G protein-coupled receptor family of SREB1, SREB2 and SREB3 is taking important roles in the living body, particularly a physiological role in the central nervous system. Also, since their amino acid sequences have a conversation ratio of 97% or more in human and rat, it is

considered that almost no interspecies differences are present regarding activities of drugs which act upon the novel G protein-coupled receptor family SREB1, SREB2 or SREB3. In consequence, when the G protein-coupled receptor protein of the invention itself or a compound or protein obtained by a screening using the receptor is developed as a medicament, the receptor has an advantage in that animal experiments using rats, for example, can be carried out in advance, prior to testing pharmacological effects on human, and is useful in terms that clinical data on human can be easily predicted from the animal experiment data.

Since expression of the G protein-coupled receptor proteins of the invention in organs and changes thereof can be detected by the method such as ELISA, radioimmunoassay, the Western blotting and the like using the antibodies, these antibodies for the novel G-protein coupled receptor proteins are useful as diagnostic agents. In addition, the antibodies capable of modifying activities of the novel G protein-coupled receptor proteins are useful as therapeutic drugs for diseases in which the novel G protein-coupled receptor proteins are involved and also as tools for the separation and purification of the receptor proteins.

## Claims

1. A G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26, or a G protein-coupled receptor protein as an equivalent to said protein.

2. A G protein-coupled receptor protein which has the amino acid sequence described in Sequence No. 2, 4, 6, 22 or 26.

3. A gene which has a nucleotide sequence coding for the G protein-coupled receptor protein described in claim 1.

4. A vector which contains the gene described in claim 3.

5. A host cell which contains the vector described in claim 4.

6. A method for producing the G protein-coupled receptor protein described in claim 1 or 2, or a G protein-coupled receptor protein as an equivalent to said protein, which comprises using the host cell described in claim 5.

7. A method for screening a medicament acting on the G protein-coupled receptor protein described in claim 1 or 2, which comprises allowing said G protein-coupled receptor protein to contact with a compound to be tested.

8. An antibody or a fragment thereof for the G protein-coupled receptor protein described in claim 1 or 2 or a partial peptide thereof.

## Abstract

This invention belongs to the genetic engineering field, and provides novel G protein-coupled receptor family proteins SREB1, SREB2 and SREB3 expressed in the central nervous system, genes coding for said proteins, vectors containing said genes, host cells containing said vectors, processes for producing said G protein-coupled receptor proteins, screening methods using said G protein-coupled receptor proteins, antibodies for said G protein-coupled receptor proteins, and screening methods using said antibodies.

Representative method for obtaining the G protein-coupled receptor proteins of the present invention:

The reverse transcriptase-polymerase chain reaction (to be referred to as RT-PCR hereinafter) is used for obtaining the G protein-coupled receptor proteins of the present invention. mRNA is extracted from human or rat brain tissue or brain-derived cells. Then, using the mRNA as the template and using two primers interposing the entire portion or a part of the G protein-coupled receptor protein translation region, RT-PCR is carried out to obtain cDNA corresponding to the G protein-coupled receptor protein or a part thereof. Then, the resulting cDNA of the novel G protein-coupled receptor protein or a part thereof



is ligated into an appropriate expression vector and expressed in a host cell to produce said G protein-coupled receptor protein.

## Abstract

This invention belongs to the genetic engineering field, and provides novel G protein-coupled receptor family proteins SREB1, SREB2 and SREB3 expressed in the central nervous system, genes coding for these proteins, screening methods using these proteins and so on.

As one of the methods for obtaining the G protein-coupled receptor proteins of the present invention, RT-PCR is carried out using mRNA extracted from human or rat brain tissue or brain-derived cells as the template and using two primers interposing the entire portion or a part of the G protein-coupled receptor protein translation region, thereby obtaining cDNA corresponding to the G protein-coupled receptor protein or a part thereof, and the cDNA is integrated into an appropriate expression vector and expressed in a host cell.

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## SEQUENCE LIST

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Variable	Mean	SD	Min	Max
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Gender	0.52	0.50	0	1
Marital status	0.65	0.48	0	1
Education	12.5	1.5	9	16
Income	15.2	5.8	10	25
Occupation	1.2	0.8	0	2
Health status	1.8	0.5	1	2
Stress level	2.5	0.7	1	3
Life satisfaction	3.2	0.6	2	4
Work-life balance	2.8	0.5	2	3
Family support	1.5	0.4	1	2
Community involvement	1.2	0.3	1	2
Volunteer work	0.8	0.2	0	1
Charitable donations	0.5	0.1	0	1
Political participation	0.3	0.1	0	1
Civic engagement	0.2	0.1	0	1
Neighborhood safety	1.5	0.4	1	2
Local government responsiveness	1.2	0.3	1	2
Public services quality	1.8	0.5	1	2
Infrastructure development	1.5	0.4	1	2
Environmental protection	1.2	0.3	1	2
Cultural heritage preservation	1.0	0.2	1	2
Historical site management	0.8	0.2	0	1
Archaeological research	0.5	0.1	0	1
Museum development	0.3	0.1	0	1
Public art installation	0.2	0.1	0	1
Urban planning	1.5	0.4	1	2
Transportation infrastructure	1.2	0.3	1	2
Park and recreation facilities	1.8	0.5	1	2
Public housing development	1.0	0.2	0	1
Community center establishment	0.8	0.2	0	1
Local business support	0.5	0.1	0	1
Entrepreneurship promotion	0.3	0.1	0	1
Job training programs	0.2	0.1	0	1
Healthcare access	1.5	0.4	1	2
Educational facilities	1.2	0.3	1	2
Public library development	0.8	0.2	0	1
Cultural institution support	0.5	0.1	0	1
Historical preservation	0.3	0.1	0	1
Archaeological excavation	0.2	0.1	0	1
Museum collection management	0.1	0.1	0	1
Public art commissioning	0.1	0.1	0	1
Urban design	1.5	0.4	1	2
Transportation infrastructure	1.2	0.3	1	2
Park and recreation facilities	1.8	0.5	1	2
Public housing development	1.0	0.2	0	1
Community center establishment	0.8	0.2	0	1
Local business support	0.5	0.1	0	1
Entrepreneurship promotion	0.3	0.1	0	1
Job training programs	0.2	0.1	0	1
Healthcare access	1.5	0.4	1	2
Educational facilities	1.2	0.3	1	2
Public library development	0.8	0.2	0	1
Cultural institution support	0.5	0.1	0	1
Historical preservation	0.3	0.1	0	1
Archaeological excavation	0.2	0.1	0	1
Museum collection management	0.1	0.1	0	1
Public art commissioning	0.1	0.1	0	1

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tca Ser	gat Asp	atc 65	ctc Ile	aga Arg	tct Ser	gca Ala	att Ile	tgt Cys	ttc Phe	cca Pro	ttt Phe	gtg Val	ttc Phe	aac Asn	tct Ser	240
gtc Val	aaa Lys	aat Asn	ggc Gly	tct Ser	acc Thr	tgg Trp	act Thr	tat Tyr	ggg Gly	act Thr	ctg Leu	act Thr	tgc Cys	aaa Lys	gtg Val	288
att Ile	gcc Ala	ttt Phe	ctg Leu	ggg Gly	ggt Val	ttg Leu	tcc Ser	tgt Cys	ttc Phe	cac His	act Thr	gct Ala	ttc Phe	atg Met	ctc Leu	336
ttc Phe	tgc Cys	atc 115	agt Ile	gtc Val	acc Thr	aga Arg	tac Tyr	tta Leu	gct Ala	atc Ile	gcc Ala	cat His	cac His	cgc Arg	ttc Phe	384
tat Tyr	aca Thr	aag Lys	agg Arg	ctg Leu	acc Thr	ttt Phe	tgg Trp	acg Thr	tgt Cys	ctg Leu	gct Ala	gtg Val	atc Ile	tgt Cys	atg Met	432
gtg Val	tgg Trp	act Thr	ctg Leu	tct Ser	gtg Val	gcc Ala	atg Met	gca Ala	ttt Phe	ccc Pro	ccg Pro	ggt Val	tta Leu	gac Asp	gtg Val	480
ggc Gly	act Thr	tac Tyr	tca Ser	ttc Phe	att Ile	agg Arg	gag Glu	gaa Glu	gat Asp	caa Gln	tgc Cys	acc Thr	ttc Phe	caa Gln	cac His	528
cgc Arg	tcc Ser	ttc Phe	agg Arg	gct Ala	aat Asn	gat Asp	tcc Ser	tta Leu	gga Gly	ttt Phe	atg Met	ctg Leu	ctt Leu	ctt Leu	gct Ala	576
ctc Leu	atc Ile	ctc 195	cta Leu	gcc Ala	aca Thr	cag Gln	ctt Leu	gtc Val	tac Tyr	ctc Leu	aag Lys	ctg Leu	ata Ile	ttt Phe	ttc Phe	624
gtc Val	cac His	gat 210	cga Arg	aga Arg	aaa Lys	atg Met	aag Lys	cca Pro	gtc Val	cag Gln	ttt Phe	gta Val	gca Ala	gca Ala	gtc Val	672
agc Ser	cag Gln	aac 225	tgg Trp	act Thr	ttt Phe	cat His	ggt Gly	cct Pro	gga Gly	gcc Ala	agt Ser	ggc Gly	cag Gln	gca Ala	gct Ala	720
gcc Ala	aat Asn	tgg 245	cta Leu	gca Ala	gga Gly	ttt Phe	gga Gly	agg Arg	ggt Gly	ccc Pro	aca Thr	cca Pro	ccc Pro	acc Thr	ttg Leu	768

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ctg ggc atc agg caa aat gca aac acc aca ggc aga aga agg cta ttg 816  
 Leu Gly Ile Arg Gln Asn Ala Asn Thr Thr Gly Arg Arg Arg Leu Leu  
 260 265 270

gtc tta gac gag ttc aaa atg gag aaa aga atc agc aga atg ttc tat 864  
 Val Leu Asp Glu Phe Lys Met Glu Lys Arg Ile Ser Arg Met Phe Tyr  
 275 280 285

ata atg act ttt ctg ttt cta acc ttg tgg ggc ccc tac ctg gtg gcc 912  
 Ile Met Thr Phe Leu Phe Leu Thr Leu Trp Gly Pro Tyr Leu Val Ala  
 290 295 300

tgt tat tgg aga gtt ttt gca aga ggg cct gta gta cca ggg gga ttt 960  
 Cys Tyr Trp Arg Val Phe Ala Arg Gly Pro Val Val Pro Gly Gly Phe  
 305 310 315 320

cta aca gct gct gtc tgg atg agt ttt gcc caa gca gga atc aat cct 1008  
 Leu Thr Ala Ala Val Trp Met Ser Phe Ala Gln Ala Gly Ile Asn Pro  
 325 330 335

ttt gtc tgc att ttc tca aac agg gag ctg agg cgc tgt ttc agc aca 1056  
 Phe Val Cys Ile Phe Ser Asn Arg Glu Leu Arg Arg Cys Phe Ser Thr  
 340 345 350

acc ctt ctt tac tgc aga aaa tcc agg tta cca agg gaa cct tac tgt 1104  
 Thr Leu Leu Tyr Cys Arg Lys Ser Arg Leu Pro Arg Glu Pro Tyr Cys  
 355 360 365

gtt ata tga 1113  
 Val Ile  
 370

<210> 4  
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 <212> PRT  
 <213> Homo sapiens

<400> 4  
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Pro Leu Thr Ala Phe Leu Lys Leu Thr Ser Leu Gly Phe Ile Ile Gly  
 20 25 30

Val Ser Val Val Gly Asn Leu Leu Ile Ser Ile Leu Leu Val Lys Asp  
 35 40 45

Lys Thr Leu His Arg Ala Pro Tyr Tyr Phe Leu Leu Asp Leu Cys Cys  
 50 55 60

Ser Asp Ile Leu Arg Ser Ala Ile Cys Phe Pro Phe Val Phe Asn Ser  
 65 70 75 80

Val Lys Asn Gly Ser Thr Trp Thr Tyr Gly Thr Leu Thr Cys Lys Val  
 85 90 95



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Ile Ala Phe Leu Gly Val Leu Ser Cys Phe His Thr Ala Phe Met Leu  
100 105 110

Phe Cys Ile Ser Val Thr Arg Tyr Leu Ala Ile Ala His His Arg Phe  
115 120 125

Tyr Thr Lys Arg Leu Thr Phe Trp Thr Cys Leu Ala Val Ile Cys Met  
130 135 140

Val Trp Thr Leu Ser Val Ala Met Ala Phe Pro Pro Val Leu Asp Val  
145 150 155 160

Gly Thr Tyr Ser Phe Ile Arg Glu Glu Asp Gln Cys Thr Phe Gln His  
165 170 175

Arg Ser Phe Arg Ala Asn Asp Ser Leu Gly Phe Met Leu Leu Leu Ala  
180 185 190

Leu Ile Leu Leu Ala Thr Gln Leu Val Tyr Leu Lys Leu Ile Phe Phe  
195 200 205

Val His Asp Arg Arg Lys Met Lys Pro Val Gln Phe Val Ala Ala Val  
210 215 220

Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Ser Gly Gln Ala Ala  
225 230 235 240

Ala Asn Trp Leu Ala Gly Phe Gly Arg Gly Pro Thr Pro Pro Thr Leu  
245 250 255

Leu Gly Ile Arg Gln Asn Ala Asn Thr Thr Gly Arg Arg Arg Leu Leu  
260 265 270

Val Leu Asp Glu Phe Lys Met Glu Lys Arg Ile Ser Arg Met Phe Tyr  
275 280 285

Ile Met Thr Phe Leu Phe Leu Thr Leu Trp Gly Pro Tyr Leu Val Ala  
290 295 300

Cys Tyr Trp Arg Val Phe Ala Arg Gly Pro Val Val Pro Gly Gly Phe  
305 310 315 320

Leu Thr Ala Ala Val Trp Met Ser Phe Ala Gln Ala Gly Ile Asn Pro  
325 330 335

Phe Val Cys Ile Phe Ser Asn Arg Glu Leu Arg Arg Cys Phe Ser Thr  
340 345 350

Thr Leu Leu Tyr Cys Arg Lys Ser Arg Leu Pro Arg Glu Pro Tyr Cys  
355 360 365

Val Ile  
370

[illegible]

<220>  
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<222> (1)..(1119)  
<223> SREB3

[illegible]

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Glu His Arg Tyr Phe Lys Ala Asn Asp Thr Leu Gly Phe Met Leu Met	
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ttg gct gtg ctc atg gca gct acc cat gct gtc tac ggc aag ctg ctc	624
Leu Ala Val Leu Met Ala Ala Thr His Ala Val Tyr Gly Lys Leu Leu	
195 200 205	
ctc ttc gag tat cgt cac cgc aag atg aag cca gtg cag atg gtg cca	672
Leu Phe Glu Tyr Arg His Arg Lys Met Lys Pro Val Gln Met Val Pro	
210 215 220	
gcc atc agc cag aac tgg aca ttc cat ggt ccc ggg gcc acc ggc cag	720
Ala Ile Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Thr Gly Gln	
225 230 235 240	
gct gct gcc aac tgg atc gcc ggc ttt ggc cgt ggg ccc atg cca cca	768
Ala Ala Ala Asn Trp Ile Ala Gly Phe Gly Arg Gly Pro Met Pro Pro	
245 250 255	
acc ctg ctg ggt atc cgg cag aat ggg cat gca gcc agc cgg cgg cta	816
Thr Leu Leu Gly Ile Arg Gln Asn Gly His Ala Ala Ser Arg Arg Leu	
260 265 270	
ctg ggc atg gac gag gtc aag ggt gaa aag cag ctg ggc cgc atg ttc	864
Leu Gly Met Asp Glu Val Lys Gly Glu Lys Gln Leu Gly Arg Met Phe	
275 280 285	
tac gcg atc aca ctg ctc ttt ctg ctc ctc tgg tca ccc tac atc gtg	912
Tyr Ala Ile Thr Leu Leu Phe Leu Leu Leu Trp Ser Pro Tyr Ile Val	
290 295 300	
gcc tgc tac tgg cga gtg ttt gtg aaa gcc tgt gct gtg ccc cac cgc	960
Ala Cys Tyr Trp Arg Val Phe Val Lys Ala Cys Ala Val Pro His Arg	
305 310 315 320	
tac ctg gcc act gct gtt tgg atg agc ttc gcc cag gct gcc gtc aac	1008
Tyr Leu Ala Thr Ala Val Trp Met Ser Phe Ala Gln Ala Ala Val Asn	
325 330 335	
cca att gtc tgc ttc ctg ctc aac aag gac ctc aag aag tgc ctg agg	1056
Pro Ile Val Cys Phe Leu Leu Asn Lys Asp Leu Lys Lys Cys Leu Arg	
340 345 350	
act cac gcc ccc tgc tgg ggc aca gga ggt gcc ccg gct ccc aga gaa	1104
Thr His Ala Pro Cys Trp Gly Thr Gly Gly Ala Pro Ala Pro Arg Glu	
355 360 365	
ccc tac tgt gtc atg tga	1122
Pro Tyr Cys Val Met	
370	

<210> 6  
 <211> 373  
 <212> PRT  
 <213> Homo sapiens

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<400> 6

Met Ala Asn Thr Thr Gly Glu Pro Glu Glu Val Ser Gly Ala Leu Ser  
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Pro Pro Ser Ala Ser Ala Tyr Val Lys Leu Val Leu Leu Gly Leu Ile  
20 25 30

Met Cys Val Ser Leu Ala Gly Asn Ala Ile Leu Ser Leu Leu Val Leu  
35 40 45

Lys Glu Arg Ala Leu His Lys Ala Pro Tyr Tyr Phe Leu Leu Asp Leu  
50 55 60

Cys Leu Ala Asp Gly Ile Arg Ser Ala Val Cys Phe Pro Phe Val Leu  
65 70 75 80

Ala Ser Val Arg His Gly Ser Ser Trp Thr Phe Ser Ala Leu Ser Cys  
85 90 95

Lys Ile Val Ala Phe Met Ala Val Leu Phe Cys Phe His Ala Ala Phe  
100 105 110

Met Leu Phe Cys Ile Ser Val Thr Arg Tyr Met Ala Ile Ala His His  
115 120 125

Arg Phe Tyr Ala Lys Arg Met Thr Leu Trp Thr Cys Ala Ala Val Ile  
130 135 140

Cys Met Ala Trp Thr Leu Ser Val Ala Met Ala Phe Pro Pro Val Phe  
145 150 155 160

Asp Val Gly Thr Tyr Lys Phe Ile Arg Glu Glu Asp Gln Cys Ile Phe  
165 170 175

Glu His Arg Tyr Phe Lys Ala Asn Asp Thr Leu Gly Phe Met Leu Met  
180 185 190

Leu Ala Val Leu Met Ala Ala Thr His Ala Val Tyr Gly Lys Leu Leu  
195 200 205

Leu Phe Glu Tyr Arg His Arg Lys Met Lys Pro Val Gln Met Val Pro  
210 215 220

Ala Ile Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Thr Gly Gln  
225 230 235 240

Ala Ala Ala Asn Trp Ile Ala Gly Phe Gly Arg Gly Pro Met Pro Pro  
245 250 255

Thr Leu Leu Gly Ile Arg Gln Asn Gly His Ala Ala Ser Arg Arg Leu  
260 265 270

Leu Gly Met Asp Glu Val Lys Gly Glu Lys Gln Leu Gly Arg Met Phe  
275 280 285

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Tyr Ala Ile Thr Leu Leu Phe Leu Leu Leu Trp Ser Pro Tyr Ile Val  
290 295 300

Ala Cys Tyr Trp Arg Val Phe Val Lys Ala Cys Ala Val Pro His Arg  
305 310 315 320

Tyr Leu Ala Thr Ala Val Trp Met Ser Phe Ala Gln Ala Ala Val Asn  
325 330 335

Pro Ile Val Cys Phe Leu Leu Asn Lys Asp Leu Lys Lys Cys Leu Arg  
340 345 350

Thr His Ala Pro Cys Trp Gly Thr Gly Gly Ala Pro Ala Pro Arg Glu  
355 360 365

Pro Tyr Cys Val Met  
370

<210> 7  
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<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Forward primer

<400> 7  
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<210> 8  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:reverse primer

<400> 8  
aaaatctaga gictatgtgg cggggcctcc c 31

<210> 9  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Forward primer

<400> 9  
aaaatctaga tctatggcga actatagcca tgca 34

<210> 10

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<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:reverse primer

<400> 10  
aaaatctaga aaggctaaag atttacagat gctcc 35

<210> 11  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Forward primer

<400> 11  
aaaatctaga giatggccaa cactaccgga gag 33

<210> 12  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:reverse primer

<400> 12  
aaaatctaga cctgtctgcc taccagcctg c 31

<210> 13  
<211> 36  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:FLAG epitope

<400> 13  
atggactaca aggacgacga tgacaagggg atcctg 36

<210> 14  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:FLAG epitope

<400> 14

Met Asp Tyr Lys Asp Asp Asp Lys Gly Ile Leu  
 1 5 10

<210> 15  
 <211> 32  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:Forward primer

<400> 15  
 aaaatctaga cggcgatggc gaacgctagt ga 32

<210> 16  
 <211> 33  
 <212> DNA  
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<220>  
 <223> Description of Artificial Sequence:reverse primer

<400> 16  
 aaaatctaga cactttgaga gtcttgtaa ggc 33

<210> 17  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:Forward primer

<400> 17  
 aaaatctaga tctatggcga actatagcca tgc 33

<210> 18  
 <211> 35  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:Forward primer

<400> 18  
 aaaatctaga aaggctaaag attacagat gctcc 35

<210> 19  
 <211> 34  
 <212> DNA  
 <213> Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:reverse primer

&lt;400&gt; 19

aaaatctaga caaatactga actggccgat cccc

34

&lt;210&gt; 20

&lt;211&gt; 34

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:reverse primer

&lt;400&gt; 20

aaaatctaga tgttgccccc agtatgtga tcat

34

&lt;210&gt; 21

&lt;211&gt; 1134

&lt;212&gt; DNA

&lt;213&gt; Rattus sp.

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(1131)

&lt;223&gt; Rat SREB1

&lt;400&gt; 21

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Met	Ala	Asn	Ala	Ser	Glu	Pro	Gly	Gly	Gly	Gly	Gly	Gly	Ala	Glu	Ala	
1				5				10					15			

gcc	gcg	ctg	ggc	ctc	agg	ctg	gcc	aca	ctc	agc	ctg	ctg	ctg	tgc	gtg	96
Ala	Ala	Leu	Gly	Leu	Arg	Leu	Ala	Thr	Leu	Ser	Leu	Leu	Leu	Cys	Val	
		20				25							30			

agc	ctg	gcg	ggc	aac	gtg	ctg	ttc	gct	ctg	ctc	atc	gtg	agg	gag	cgc	144
Ser	Leu	Ala	Gly	Asn	Val	Leu	Phe	Ala	Leu	Leu	Ile	Val	Arg	Glu	Arg	
		35				40					45					

agc	ctg	cac	cgc	gcg	cct	tac	tac	ctg	ctg	ctc	gac	ctg	tgc	ctg	gcc	192
Ser	Leu	His	Arg	Ala	Pro	Tyr	Tyr	Leu	Leu	Leu	Asp	Leu	Cys	Leu	Ala	
		50				55					60					

gac	ggg	ctg	cgc	gcg	ctc	gcc	tgt	ctc	ccg	gcc	gtc	atg	ctg	gct	gcg	240
Asp	Gly	Leu	Arg	Ala	Leu	Ala	Cys	Leu	Pro	Ala	Val	Met	Leu	Ala	Ala	
65				70				75					80			

cgg	cgc	gcg	gca	gcc	gcg	gcg	ggg	acg	cct	ccg	ggt	gcg	ctg	ggc	tgc	288
Arg	Arg	Ala	Ala	Ala	Ala	Ala	Gly	Thr	Pro	Pro	Gly	Ala	Leu	Gly	Cys	
				85				90					95			

aag	ctg	ctg	gcc	ttc	ctg	gcc	gcg	ctc	ttc	tgc	ttc	cac	gcg	gcc	ttc	336
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----



Lys	Leu	Leu	Ala	Phe	Leu	Ala	Ala	Leu	Phe	Cys	Phe	His	Ala	Ala	Phe		
			100				105						110				
ctg	ctg	ctg	ggc	gig	ggc	gtc	acc	cgc	tac	ctg	gcc	atc	gct	cac	cac	384	
Leu	Leu	Leu	Gly	Val	Gly	Val	Thr	Arg	Tyr	Leu	Ala	Ile	Ala	His	His		
			115				120				125						
cgc	ttc	tat	gcc	gag	cgc	ctg	gcc	ggc	tgg	ccg	tgc	gcc	gcg	atg	ctg	432	
Arg	Phe	Tyr	Ala	Glu	Arg	Leu	Ala	Gly	Trp	Pro	Cys	Ala	Ala	Met	Leu		
			130				135				140						
gtg	tgc	gcc	gcc	tgg	gcg	ctg	gct	tig	gcc	gcg	gcc	ttc	ccg	ccg	gtg	480	
Val	Cys	Ala	Ala	Trp	Ala	Leu	Ala	Leu	Ala	Ala	Ala	Phe	Pro	Pro	Val		
			145				150				155				160		
ctg	gac	ggc	ggt	ggc	gcg	gac	gac	gag	gat	gcg	ccg	tgc	gcc	ctg	gag	528	
Leu	Asp	Gly	Gly	Gly	Ala	Asp	Asp	Glu	Asp	Ala	Pro	Cys	Ala	Leu	Glu		
			165				170				175						
cag	cgg	ccc	gac	ggc	gcc	ccg	ggt	gcg	cta	ggc	ttc	ctg	ctg	ctc	ctg	576	
Gln	Arg	Pro	Asp	Gly	Ala	Pro	Gly	Ala	Leu	Gly	Phe	Leu	Leu	Leu	Leu		
			180				185				190						
gcc	gcg	gtg	gtg	ggc	gcc	acg	cac	ctc	gtc	tac	ctt	cgc	ctg	ctc	ttc	624	
Ala	Ala	Val	Val	Gly	Ala	Thr	His	Leu	Val	Tyr	Leu	Arg	Leu	Leu	Phe		
			195				200				205						
ttc	atc	cac	gac	cgc	cgc	aag	atg	cgg	ccc	gca	cgc	ctg	gtg	ccc	gcc	672	
Phe	Ile	His	Asp	Arg	Arg	Lys	Met	Arg	Pro	Ala	Arg	Leu	Val	Pro	Ala		
			210				215				220						
gtc	agc	cac	gac	tgg	acc	ttc	cac	ggc	ccg	ggc	gcc	acc	ggt	caa	gcg	720	
Val	Ser	His	Asp	Trp	Thr	Phe	His	Gly	Pro	Gly	Ala	Thr	Gly	Gln	Ala		
			225				230				235				240		
gcc	gcc	aac	tgg	acg	gcg	ggc	ttc	ggc	cgc	ggg	ccc	acg	cca	cct	gcg	768	
Ala	Ala	Asn	Trp	Thr	Ala	Gly	Phe	Gly	Arg	Gly	Pro	Thr	Pro	Pro	Ala		
			245				250				255						
ctc	gtg	ggc	atc	agg	cct	gca	ggc	ccg	ggc	cgc	gga	gcc	cgg	cgc	ctc	816	
Leu	Val	Gly	Ile	Arg	Pro	Ala	Gly	Pro	Gly	Arg	Gly	Ala	Arg	Arg	Leu		
			260				265				270						
ctg	gtg	ctg	gag	gaa	ttc	aag	acg	gag	aag	agg	ctg	tgc	aag	atg	ttc	864	
Leu	Val	Leu	Glu	Glu	Phe	Lys	Thr	Glu	Lys	Arg	Leu	Cys	Lys	Met	Phe		
			275				280				285						
tac	gcc	atc	acg	ctg	ctc	ttc	ctg	ctc	ctc	tgg	ggg	ccc	tat	gtg	gtt	912	
Tyr	Ala	Ile	Thr	Leu	Leu	Phe	Leu	Leu	Leu	Trp	Gly	Pro	Tyr	Val	Val		
			290				295				300						
gcc	agt	tac	ctg	cgc	gtc	ctg	gtg	cgg	ccc	gga	gct	gtc	ccg	cag	gcc	960	
Ala	Ser	Tyr	Leu	Arg	Val	Leu	Val	Arg	Pro	Gly	Ala	Val	Pro	Gln	Ala		
			305				310				315				320		
tac	ctg	aca	gcc	tcg	gtg	tgg	ctg	aca	ttc	gca	cag	gcc	ggc	atc	aac	1008</	

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1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	

Gln Arg Pro Asp Gly Ala Pro Gly Ala Leu Gly Phe Leu Leu Leu Leu

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180	185	190
Ala Ala Val Val Gly Ala Thr His	Leu Val Tyr Leu Arg Leu Leu Phe	
195	200	205
Phe Ile His Asp Arg Arg Lys Met Arg Pro Ala Arg Leu Val Pro Ala		
210	215	220
Val Ser His Asp Trp Thr Phe His Gly Pro Gly Ala Thr Gly Gln Ala		
225	230	235
Ala Ala Asn Trp Thr Ala Gly Phe Gly Arg Gly Pro Thr Pro Pro Ala		
245	250	255
Leu Val Gly Ile Arg Pro Ala Gly Pro Gly Arg Gly Ala Arg Arg Leu		
260	265	270
Leu Val Leu Glu Glu Phe Lys Thr Glu Lys Arg Leu Cys Lys Met Phe		
275	280	285
Tyr Ala Ile Thr Leu Leu Phe Leu Leu Leu Trp Gly Pro Tyr Val Val		
290	295	300
Ala Ser Tyr Leu Arg Val Leu Val Arg Pro Gly Ala Val Pro Gln Ala		
305	310	315
Tyr Leu Thr Ala Ser Val Trp Leu Thr Phe Ala Gln Ala Gly Ile Asn		
325	330	335
Pro Val Val Cys Phe Leu Phe Asn Arg Glu Leu Arg Asp Cys Phe Arg		
340	345	350
Ala Gln Phe Pro Cys Cys Gln Ser Pro Gln Ala Thr Gln Ala Thr Leu		
355	360	365
Pro Cys Asp Leu Lys Gly Ile Gly Leu		
370	375	

<210> 23  
 <211> 1113  
 <212> DNA  
 <213> Rattus sp.

<220>  
 <221> CDS  
 <222> (1)..(1110)  
 <223> Rat SREB2

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Met Ala Asn Tyr Ser His Ala Ala Asp Asn Ile Leu Gln Asn Leu Ser	
1 5 10 15	
cct cta aca gcc ttt ctg aaa ctg act tcc ttg ggt ttc ata ata gga	96
Pro Leu Thr Ala Phe Leu Lys Leu Thr Ser Leu Gly Phe Ile Ile Gly	

20										25					30					
gtc	agt	gtg	gtg	ggc	aac	ctt	ctg	atc	tcc	att	ttg	cta	gtg	aaa	gat	144				
Val	Ser	Val	Val	Gly	Asn	Leu	Leu	Ile	Ser	Ile	Leu	Leu	Val	Lys	Asp					
35				40				45												
aag	acc	ttg	cat	aga	gct	cct	tac	tac	ttc	ctg	ctg	gat	ctg	tgc	tgc	192				
Lys	Thr	Leu	His	Arg	Ala	Pro	Tyr	Tyr	Phe	Leu	Leu	Asp	Leu	Cys	Cys					
50				55				60												
tca	gac	atc	ctc	aga	tct	gca	att	tgt	ttt	cca	ttt	gta	ttc	aac	tct	240				
Ser	Asp	Ile	Leu	Arg	Ser	Ala	Ile	Cys	Phe	Pro	Phe	Val	Phe	Asn	Ser					
65				70				75				80								
gtc	aaa	aat	ggc	tct	acc	tgg	act	tac	ggg	act	ctg	act	tgc	aaa	gtg	288				
Val	Lys	Asn	Gly	Ser	Thr	Trp	Thr	Tyr	Gly	Thr	Leu	Thr	Cys	Lys	Val					
85				90				95												
att	gcc	ttt	ctg	ggg	gtt	tig	tcc	tgt	ttc	cac	act	gcc	ttc	atg	ctc	336				
Ile	Ala	Phe	Leu	Gly	Val	Leu	Ser	Cys	Phe	His	Thr	Ala	Phe	Met	Leu					
100				105				110												
ttc	tgc	atc	agc	gtc	acc	aga	tac	tta	gcc	atc	gcc	cat	cac	cgc	ttc	384				
Phe	Cys	Ile	Ser	Val	Thr	Arg	Tyr	Leu	Ala	Ile	Ala	His	His	Arg	Phe					
115				120				125												
tat	aca	aag	agg	ctg	acc	ttt	tgg	acg	tgt	tig	gct	gtg	atc	tgc	atg	432				
Tyr	Thr	Lys	Arg	Leu	Thr	Phe	Trp	Thr	Cys	Leu	Ala	Val	Ile	Cys	Met					
130				135				140												
gtg	tgg	act	ctg	tct	gtg	gcc	atg	gca	ttt	ccc	cca	gtt	tta	gat	gta	480				
Val	Trp	Thr	Leu	Ser	Val	Ala	Met	Ala	Phe	Pro	Pro	Val	Leu	Asp	Val					
145				150				155				160								
ggc	acc	tac	tca	ttc	att	agg	gag	gag	gat	cag	igt	acc	ttc	caa	cac	528				
Gly	Thr	Tyr	Ser	Phe	Ile	Arg	Glu	Glu	Asp	Gln	Cys	Thr	Phe	Gln	His					
165				170				175												
cgc	tcc	ttc	agg	gct	aac	gat	tcc	cta	gga	ttt	atg	ctg	ctc	ctt	gct	576				
Arg	Ser	Phe	Arg	Ala	Asn	Asp	Ser	Leu	Gly	Phe	Met	Leu	Leu	Leu	Ala					
180				185				190												
ctc	atc	ctc	cta	gcc	aca	cag	ctt	gtc	tac	ctc	aag	ctg	ata	ttt	ttt	624				
Leu	Ile	Leu	Leu	Ala	Thr	Gln	Leu	Val	Tyr	Leu	Lys	Leu	Ile	Phe	Phe					
195				200				205												
gtc	cac	gat	cga	agg	aaa	atg	aag	cca	gtc	cag	ttt	gta	gca	gca	gtg	672				
Val	His	Asp	Arg	Arg	Lys	Met	Lys	Pro	Val	Gln	Phe	Val	Ala	Ala	Val					
210				215				220												
agt	cag	aac	tgg	acc	ttt	cat	ggc	cct	gga	gct	agt	ggc	cag	gca	gct	720				
Ser	Gln	Asn	Trp	Thr	Phe	His	Gly	Pro	Gly	Ala	Ser	Gly	Gln	Ala	Ala					
225				230				235				240								
gcc	aat	tgg	cta	gca	gga	ttt	gga	agg	ggt	ccc	aca	cca	ccc	acc	tig	768				
Ala	Asn	Trp	Leu	Ala	Gly	Phe	Gly	Arg	Gly	Pro	Thr	Pro	Pro	Thr	Leu					

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Leu Gly Ile Arg Gln Asn Ala Asn Thr Thr Gly Arg Arg Arg Leu Leu			
260	265	270	
gtt ttg gat gag ttc aaa atg gag aaa aga atc agc aga atg ttc tat			864
Val Leu Asp Glu Phe Lys Met Glu Lys Arg Ile Ser Arg Met Phe Tyr			
275	280	285	
ata atg act ttc ctc ttc cta acc tgg tgg ggt ccc tac ctg gtg gcc			912
Ile Met Thr Phe Leu Phe Leu Thr Leu Trp Gly Pro Tyr Leu Val Ala			
290	295	300	
tgc tat tgg aga gtt ttt gca aga ggg cct gta gta cca ggg gga ttt			960
Cys Tyr Trp Arg Val Phe Ala Arg Gly Pro Val Val Pro Gly Gly Phe			
305	310	315	320
cta aca gcc gct gtc tgg atg agt ttc gcc caa gca gga atc aat ccc			1008
Leu Thr Ala Ala Val Trp Met Ser Phe Ala Gln Ala Gly Ile Asn Pro			
325	330	335	
ttt gtc tgc att ttc tcc aac agg gag ctg agg cgc tgt ttc agc aca			1056
Phe Val Cys Ile Phe Ser Asn Arg Glu Leu Arg Arg Cys Phe Ser Thr			
340	345	350	
acc ctt ctt tac tgc aga aaa tcc agg tta cca agg gaa cct tac tgt			1104
Thr Leu Leu Tyr Cys Arg Lys Ser Arg Leu Pro Arg Glu Pro Tyr Cys			
355	360	365	
gtt ata tga			1113
Val Ile			
370			

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 Val Ser Val Val Gly Asn Leu Leu Ile Ser Ile Leu Leu Val Lys Asp  
 35 40 45  
 Lys Thr Leu His Arg Ala Pro Tyr Tyr Phe Leu Leu Asp Leu Cys Cys  
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[illegible]

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 Met Ala Asn Thr Thr Gly Glu Pro Glu Glu Val Ser Gly Ala Leu Ser  
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 Leu Pro Ser Ala Ser Ala Tyr Val Lys Leu Val Leu Leu Gly Leu Ile  
 20 25 30

atg tgt gta agc ctg gca ggc aat gcc atc ttg tcc ctg ctg gtg ctc 144  
 Met Cys Val Ser Leu Ala Gly Asn Ala Ile Leu Ser Leu Leu Val Leu  
 35 40 45

aag gag cgt gcc ctg cac aag gct cct tac tac ttt ctg ctg gac ctg 192  
 Lys Glu Arg Ala Leu His Lys Ala Pro Tyr Tyr Phe Leu Leu Asp Leu  
 50 55 60

tgc cta gcc gat ggc ata cgc tct gcc atc tgc ttc ccc ttt gta ctg 240  
 Cys Leu Ala Asp Gly Ile Arg Ser Ala Ile Cys Phe Pro Phe Val Leu  
 65 70 75 80

gct tct gtg cgc cat ggc tcc tgc tgg acc ttc agt gca ctc agc tgt 288  
 Ala Ser Val Arg His Gly Ser Ser Trp Thr Phe Ser Ala Leu Ser Cys  
 85 90 95

aag att gtg gcc ttt atg gct gtg ctc ttt tgc ttc cat gcg gcc ttc 336  
 Lys Ile Val Ala Phe Met Ala Val Leu Phe Cys Phe His Ala Ala Phe  
 100 105 110

atg ctg ttc tgc atc agc gtc acc cgc tac atg gcc atc gcc cac cac 384  
 Met Leu Phe Cys Ile Ser Val Thr Arg Tyr Met Ala Ile Ala His His  
 115 120 125

cgc ttc tat gcc aag cgc atg aca ctc tgg aca tgc gca gct gtc atc 432  
 Arg Phe Tyr Ala Lys Arg Met Thr Leu Trp Thr Cys Ala Ala Val Ile  
 130 135 140

tgc atg gcc tgg acc ttg tct gtg gcc atg gct ttc cca cct gtc ttt 480  
 Cys Met Ala Trp Thr Leu Ser Val Ala Met Ala Phe Pro Pro Val Phe  
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gat gtg ggc acc tac aag ttt atc cga gag gag gac cag tgc atc ttt 528  
 Asp Val Gly Thr Tyr Lys Phe Ile Arg Glu Glu Asp Gln Cys Ile Phe  
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gag cat cgc tac ttc aaa gca aat gac act ctg ggc ttt atg ctt atg 576  
 Glu His Arg Tyr Phe Lys Ala Asn Asp Thr Leu Gly Phe Met Leu Met  
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ttg gct gtg ctc atg gca gcc aca cat gct gtc tat ggc aag ctg cta 624  
 Leu Ala Val Leu Met Ala Ala Thr His Ala Val Tyr Gly Lys Leu Leu  
 195 200 205

ctc ttc gag tat cgt cac cgc aag atg aag cca gtg cag atg gtg ccc 672  
 Leu Phe Glu Tyr Arg His Arg Lys Met Lys Pro Val Gln Met Val Pro  
 210 215 220

gcc atc agc caa aac tgg aca ttc cat ggc cct ggg gct acc ggc cag 720  
 Ala Ile Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Thr Gly Gln  
 225 230 235 240

gct gct gcc aac tgg atc gct ggc ttt ggc cgt ggg ccc atg cca cca 768  
 Ala Ala Ala Asn Trp Ile Ala Gly Phe Gly Arg Gly Pro Met Pro Pro  
 245 250 255

act ctg ctg ggt atc cgg cag aat ggg cat gca gct agc cgg cgg cta 816  
 Thr Leu Leu Gly Ile Arg Gln Asn Gly His Ala Ala Ser Arg Arg Leu  
 260 265 270

ctg ggc atg gac gag gtc aag ggt gaa aag cag ctg ggc cga atg ttc 864  
 Leu Gly Met Asp Glu Val Lys Gly Glu Lys Gln Leu Gly Arg Met Phe  
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 Tyr Ala Ile Thr Leu Leu Phe Leu Leu Leu Trp Ser Pro Tyr Ile Val  
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gcc tgc tac tgg cga gtg ttt gtg aaa gcc tgc gct gtg ccc cac cgc 960  
 Ala Cys Tyr Trp Arg Val Phe Val Lys Ala Cys Ala Val Pro His Arg  
 305 310 315 320

tac ctg gcc act gct gtt tgg atg agc ttc gcc cag gct gct gtc aac 1008  
 Tyr Leu Ala Thr Ala Val Trp Met Ser Phe Ala Gln Ala Ala Val Asn  
 325 330 335

cca atc gtc tgc ttc ctg ctt aac aag gac ctc aag aag tgc ctg agg 1056  
 Pro Ile Val Cys Phe Leu Leu Asn Lys Asp Leu Lys Lys Cys Leu Arg  
 340 345 350

act cat gcc cct tgc tgg ggc aca gga ggt gcc cca gct ccc aga gaa 1104  
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Leu Pro Ser Ala Ser Ala Tyr Val Lys Leu Val Leu Leu Gly Leu Ile  
20 25 30

Met Cys Val Ser Leu Ala Gly Asn Ala Ile Leu Ser Leu Leu Val Leu  
35 40 45

Lys Glu Arg Ala Leu His Lys Ala Pro Tyr Tyr Phe Leu Leu Asp Leu  
50 55 60

Cys Leu Ala Asp Gly Ile Arg Ser Ala Ile Cys Phe Pro Phe Val Leu  
65 70 75 80

Ala Ser Val Arg His Gly Ser Ser Trp Thr Phe Ser Ala Leu Ser Cys  
85 90 95

Lys Ile Val Ala Phe Met Ala Val Leu Phe Cys Phe His Ala Ala Phe  
100 105 110

Met Leu Phe Cys Ile Ser Val Thr Arg Tyr Met Ala Ile Ala His His  
115 120 125

Arg Phe Tyr Ala Lys Arg Met Thr Leu Trp Thr Cys Ala Ala Val Ile  
130 135 140

Cys Met Ala Trp Thr Leu Ser Val Ala Met Ala Phe Pro Pro Val Phe  
145 150 155 160

Asp Val Gly Thr Tyr Lys Phe Ile Arg Glu Glu Asp Gln Cys Ile Phe  
165 170 175

Glu His Arg Tyr Phe Lys Ala Asn Asp Thr Leu Gly Phe Met Leu Met  
180 185 190

Leu Ala Val Leu Met Ala Ala Thr His Ala Val Tyr Gly Lys Leu Leu  
195 200 205

Leu Phe Glu Tyr Arg His Arg Lys Met Lys Pro Val Gln Met Val Pro  
210 215 220

Ala Ile Ser Gln Asn Trp Thr Phe His Gly Pro Gly Ala Thr Gly Gln  
225 230 235 240

Ala Ala Ala Asn Trp Ile Ala Gly Phe Gly Arg Gly Pro Met Pro Pro  
245 250 255

Thr Leu Leu Gly Ile Arg Gln Asn Gly His Ala Ala Ser Arg Arg Leu  
260 265 270

Leu Gly Met Asp Glu Val Lys Gly Glu Lys Gln Leu Gly Arg Met Phe

285

Pro Tyr Cys Val Met  
370

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FIG. 1

SREB 1 MANAS E P G G S G G G E A A A L G - - - L K L A T L S L L L C V S L A G N 36  
 SREB 2 M A N Y S H A A D N I L Q N L S P - - L T A F L K L T S L G F I I G V S V V G N 38  
 SREB 3 M A N T T G E P E E V S G A L S P P S A S A Y V K L V L L G L I M C V S L A G N 40

SREB 1 V L F A L L I V R E R S L H R A P Y Y L L D L C L A D G L R A L A C L P A V M 76  
 SREB 2 L L I S I L L V K D K T L H R A P Y Y F L L D L C C S D I L R S A I C F P F V F 78  
 SREB 3 A I L S L L V L K E R A L H K A P Y Y F L L D L C L A D G I R S A V C F P F V L 80

SREB 1 L A A R R A A A A A G A P P G A L G C K L L A F L A A L F C F H A A F L L L G V 116  
 SREB 2 N S V K N G S T W T Y - - - G T L T C K V I A F L G V L S C F H T A F M L F C I 115  
 SREB 3 A S V R H G S S W T F - - - S A L S C K I V A F M A V L F C F H A A F M L F C I 117

SREB 1 G V T R Y L A I A H H R F Y A E R L A G W P C A A M L V C A A W A L A L A A A F 156  
 SREB 2 S V T R Y L A I A H H R F Y T K R L T F W T C L A V - I C M V W T L S V A M A F 154  
 SREB 3 S V T R Y M A I A H H R F Y A K R M T L W T C A A V - I C M A W T L S V A M A F 156

SREB 1 P P V L D G G G - - - D D E D A P C A L E Q R P D G A P G A L G F L L L L A V V 193  
 SREB 2 P P V L D V G T Y S F I R E E D O C T F Q H R S F R A N D S L G F M L L L A L I 194  
 SREB 3 P P V F D V G T Y K F I R E E D O C I F E H R Y F K A N D T L G F M L M L A V L 196

SREB 1 V G A T H L V Y L R L L F F I H D R R K M R P A R L V P A V S H D W T F H G P G 233  
 SREB 2 L L A T Q L V Y L K L I F F V H D R R K M K P V Q F V A A V S O N W T F H G P G 234  
 SREB 3 M A A T H A V Y G K L L L F E Y R H R K M K P V Q M V P A I S O N W T F H G P G 236

SREB 1 A T G Q A A A N W T A G E G R G P T P P A L V G I R P A G P G R G A R R L L V L 273  
 SREB 2 A S G Q A A A N W L A G E G R G P T P P T L L G I R O N A N T T G R R L L V L 274  
 SREB 3 A T G Q A A A N W I A G E G R G P M P T L L G I R O N G H A A S R R - L L G M 275

SREB 1 E E F K T E K R L C K M F Y A V T L L F L L L W G P Y V V A S Y L R V L V R P G 313  
 SREB 2 D E F K M E K R I S R M F Y I M T L F L T L W G P Y L V A C Y W R V F A R G P 314  
 SREB 3 D E V K G E K Q L G R M F Y A I T L L F L L L W S P Y I V A C Y W R V F V K A C 315

SREB 1 A V P Q A Y L T A S V W L T F A Q A G I N P V V C F L F N R E L R D C F R A Q F 353  
 SREB 2 V V P G G F L T A A V W M S F A Q A G I N P F V C I F S N R E L R C F S T L 354  
 SREB 3 A V P H R Y L A T A V W M S F A Q A A V N P I V C F L L N K D L K K C L R T H A 355

SREB 1 E C C Q S P R T T Q A T H P - - C D L K G I G L . 376  
 SREB 2 L Y C R K S - - - R L P R E P Y C - - - - V I . 371  
 SREB 3 E - C W G T G G A P A P R E P Y C - - - - V M . 374

004730-00000000

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FIG. 2

9.5-  
7.5-  
4.4-  
2.4-  
1.4-

heart  
brain  
placenta  
lung  
liver  
skeletal muscle  
kidney  
pancreas

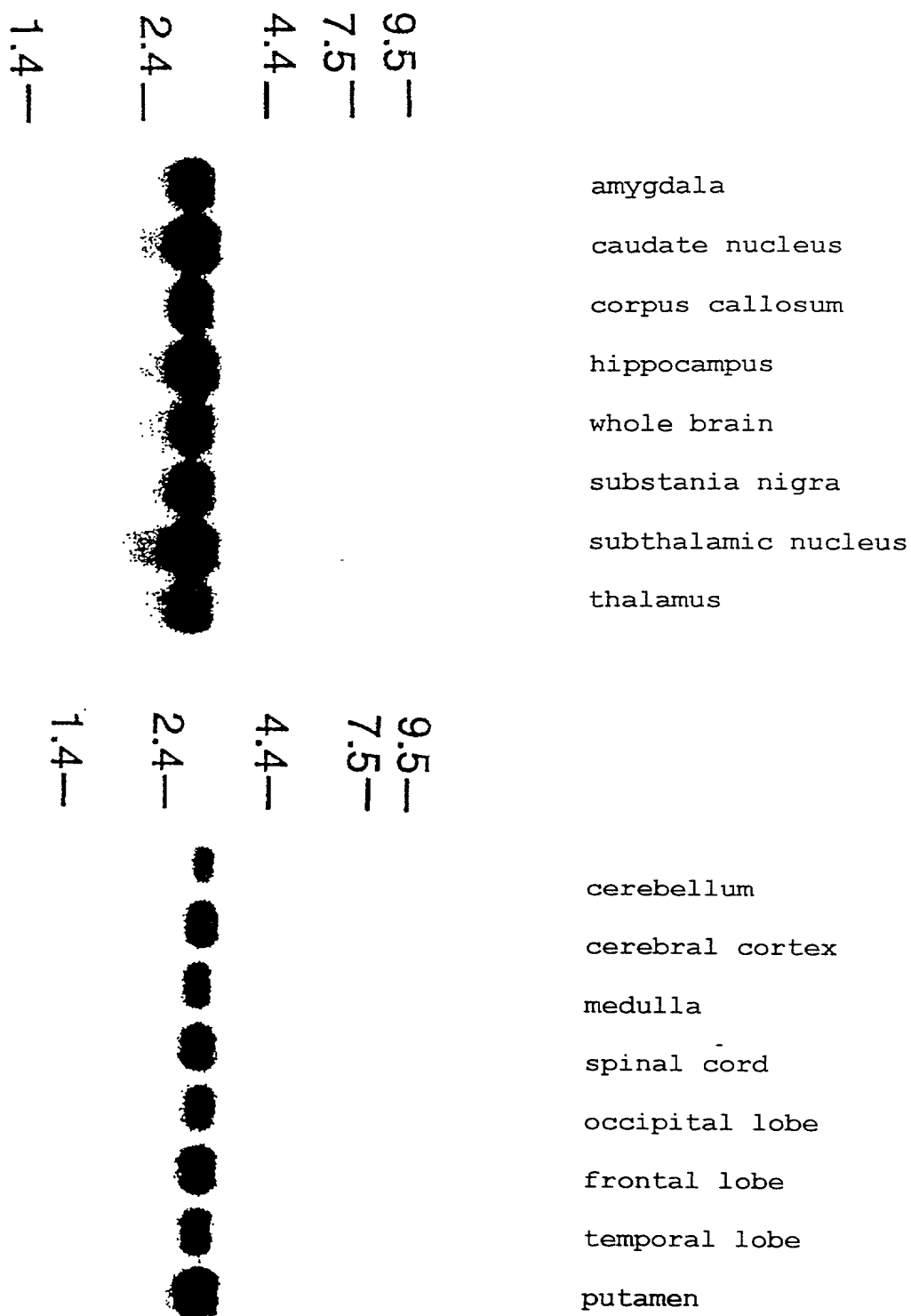
9.5-  
7.5-  
4.4-  
2.4-  
1.4-

spleen  
thymus  
prostate  
testis  
ovary  
small intestine  
large intestine  
peripheral leukocyte

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FIG. 3



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FIG. 4

9.5-  
7.5-  
4.4-  
2.4-  
1.4-



heart  
brain  
placenta  
lung  
liver  
skeletal muscle  
kidney  
pancreas

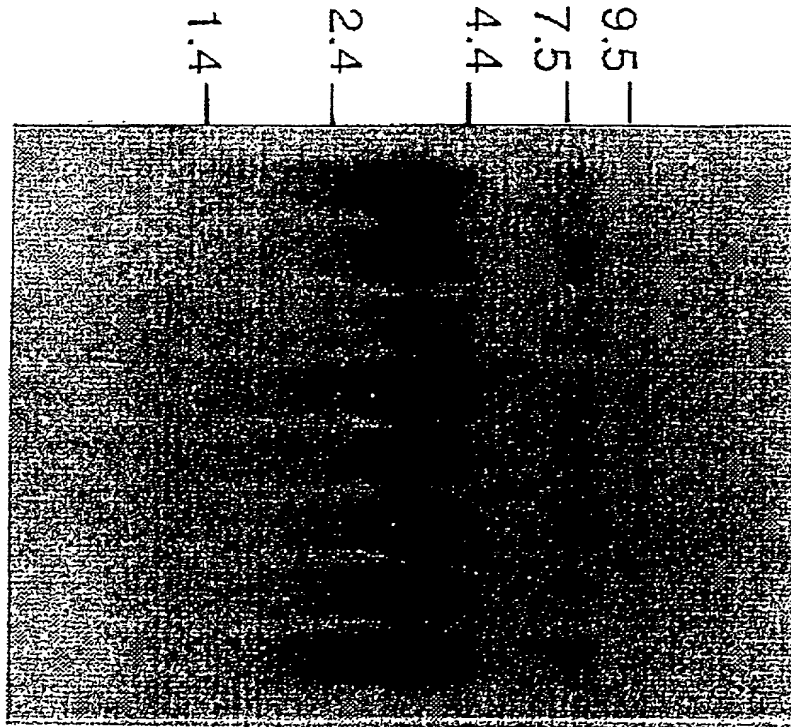
9.5-  
7.5-  
4.4-  
2.4-  
1.4-



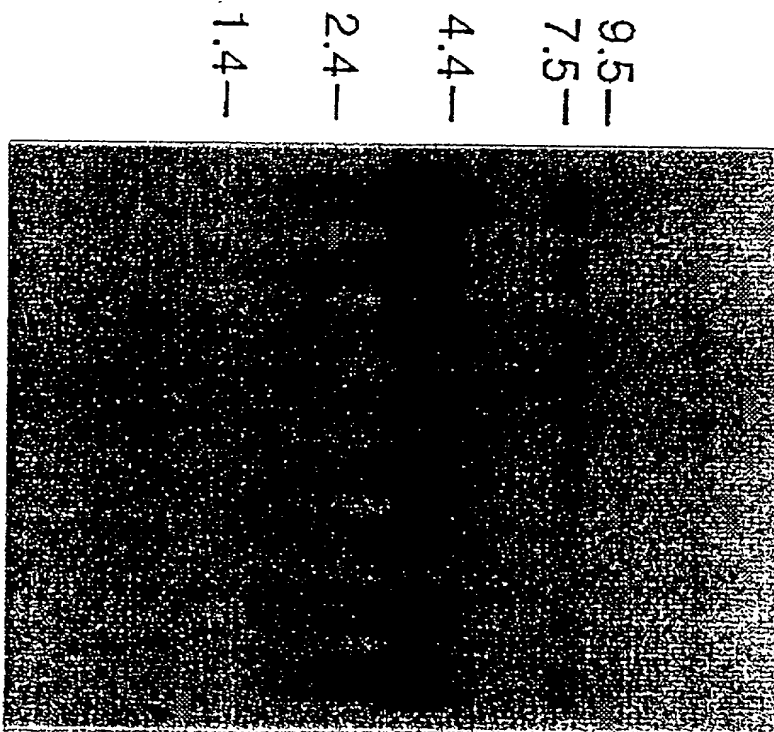
spleen  
thymus  
prostate  
testis  
ovary  
small intestine  
large intestine  
peripheral leukocyte

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FIG. 5



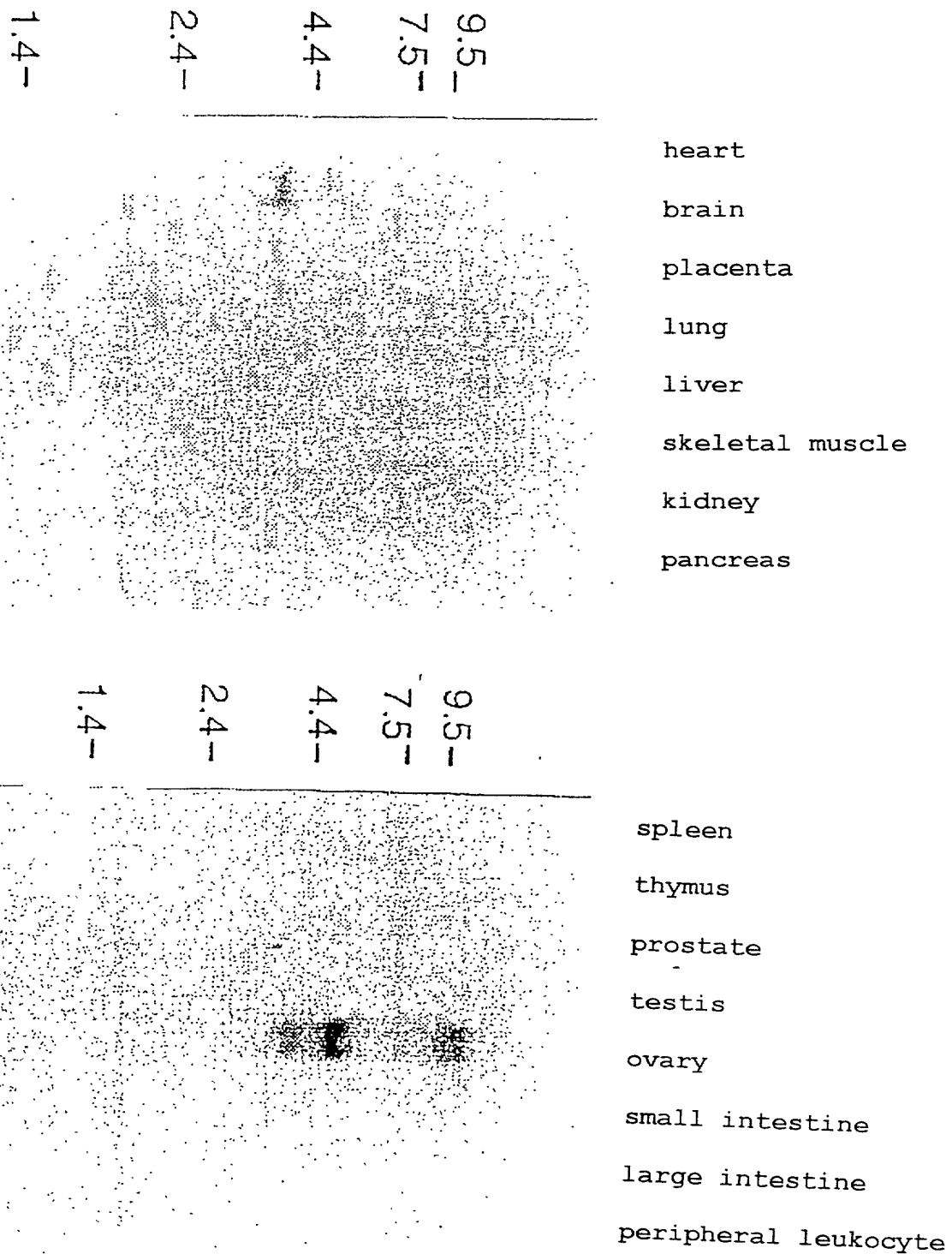
amygdala  
caudate nucleus  
corpus callosum  
hippocampus  
whole brain  
substantia nigra  
subthalamic nucleus  
thalamus



cerebellum  
cerebral cortex  
medulla  
spinal cord  
occipital lobe  
frontal lobe  
temporal lobe  
putamen

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FIG. 6

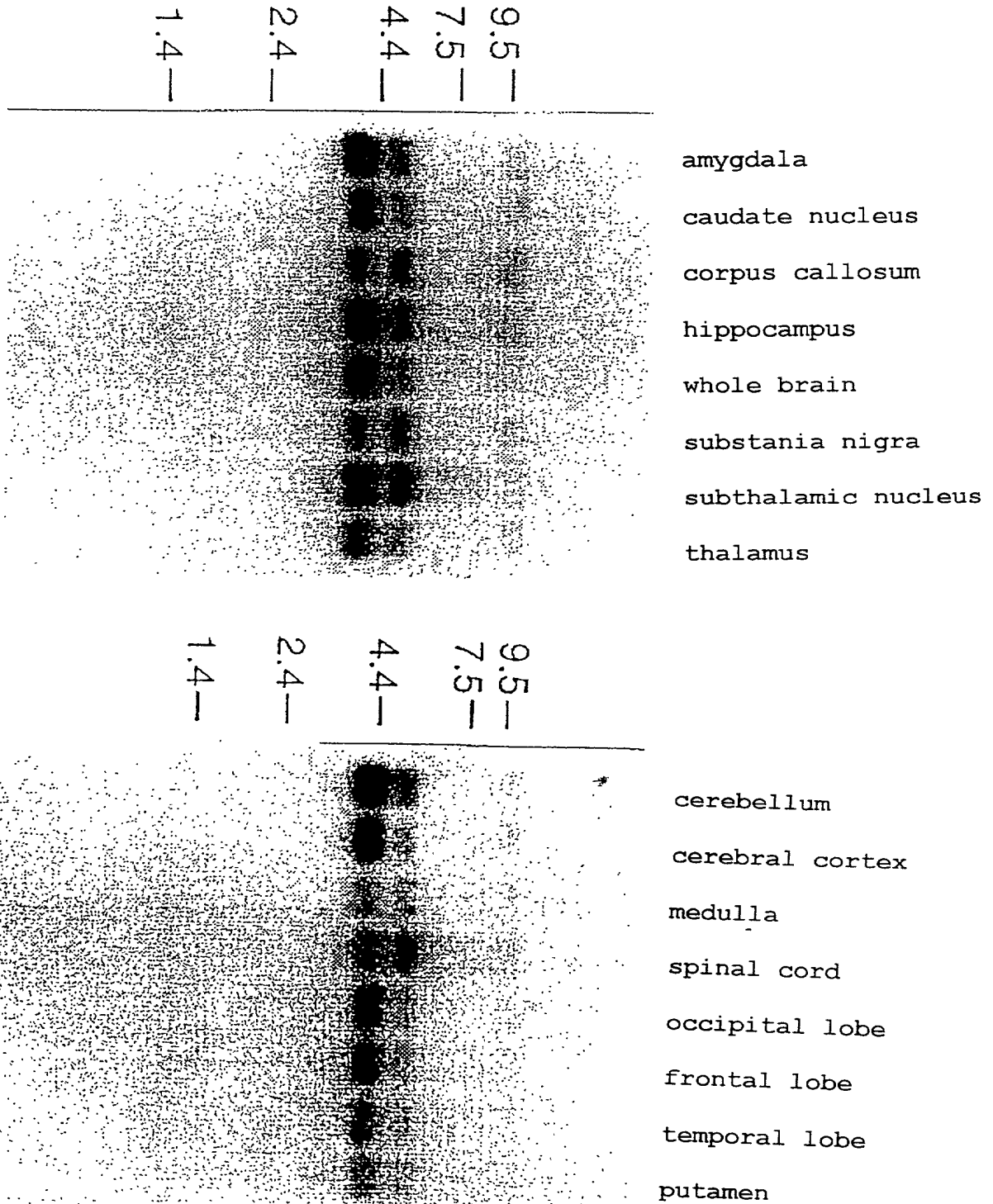


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FIG. 7



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FIG. 8

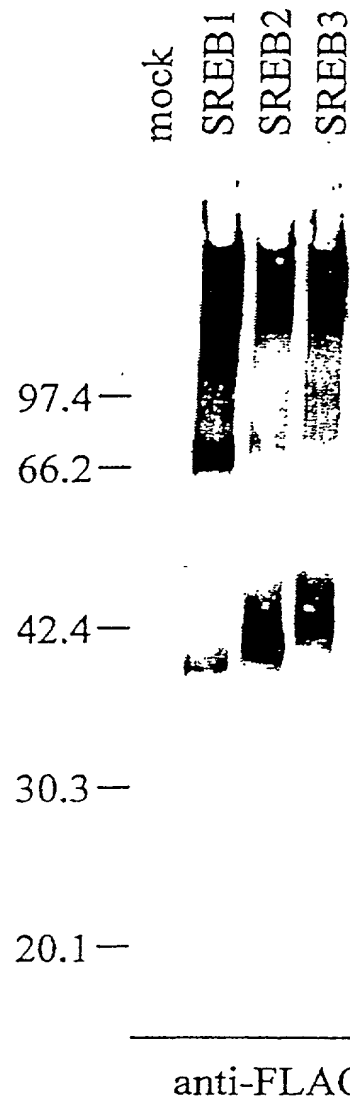


FIG. 9

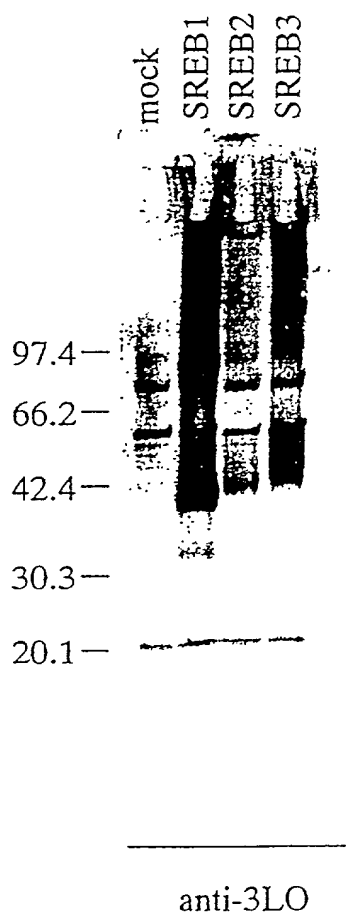
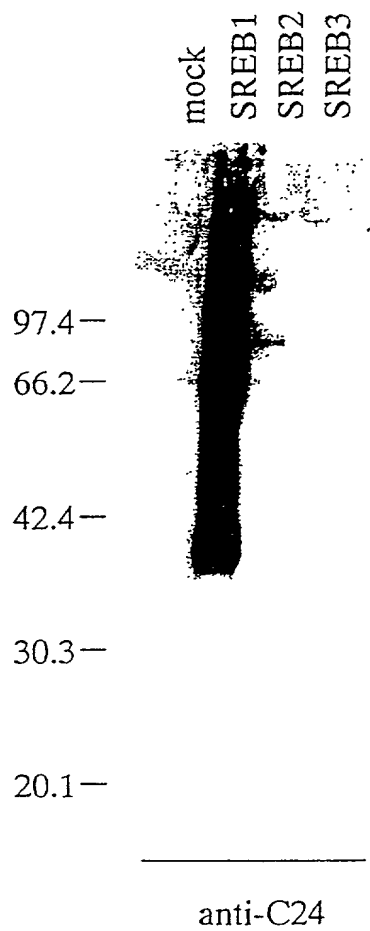


FIG. 10



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FIG. 11

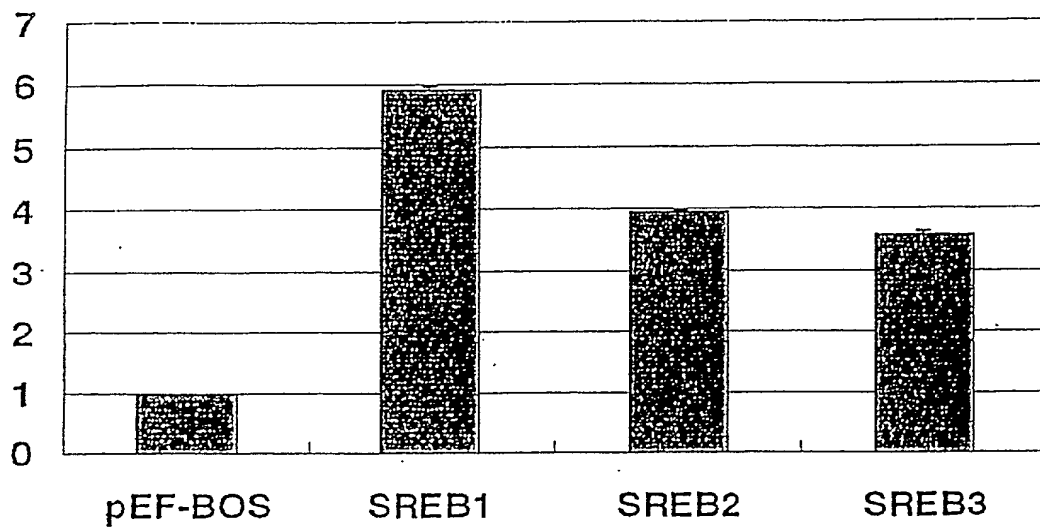
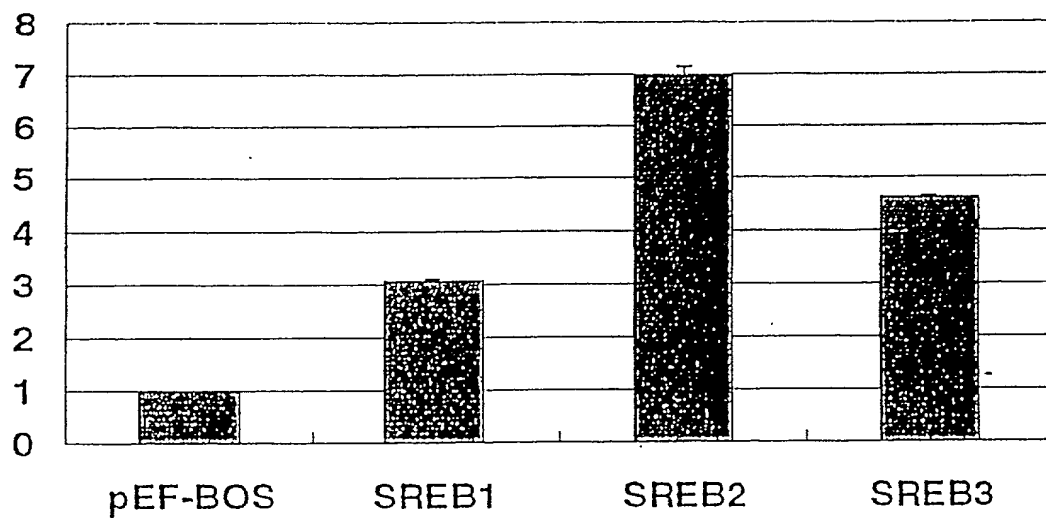


FIG. 12



# DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Novel G Protein-coupled Receptor Proteins

the specification of which is attached hereto unless the following box is checked:

☒ was filed on March 11, 1999 as United States Application Number or PCT International Application Number PCT/JP99/01191 and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information of which is material to the patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
<u>P.Hei.10-060245</u>	<u>Japan</u>	<u>12/March/1998</u>	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	
<u>P.Hei.11-026774</u>	<u>Japan</u>	<u>03/February/1999</u>	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	
_____	_____	_____	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefits under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application Number)

(Filing Date)

I hereby claim the benefits under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application Number)

(Filing Date)

(Status - patented, pending, abandoned)

(Application Number)

(Filing Date)

(Status - patented, pending, abandoned)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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